Division of Agricultural Sciences
UNIVERSITY OF CALIFORNIA

The AUTORADIOGRAPHY of PLANT MATERIALS

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Unless otherwise noted, all illustrations of mounted plants and their autographs in this publication show autographs on top and mounts below.

JUNE, 1964

PREFACE

Plant autoradiography is a new and revolutionary scientific technique, and the authors of this publication are proud and grateful that they have been able to contribute to it by developing the freeze-drying method of plant preparation and by discovering refinements in technique that make critical studies on intact plants possible. The ability to study distribution patterns of labeled tracers in such plants has resulted in research of outstanding importance, and it is these techniques and studies which are the subject of the present volume.

The work described herein provides strong evidence for a mass-flow type of mechanism to explain food and tracer movement in plants. This evidence, together with the new anatomical information furnished by electron microscopy, provides a picture of the mechanics of solute and water movement in plants as illuminating to botanists as were the data supplied to animal workers by the blood circulation studies instituted by Harvey and extended by others.

Botanists have struggled for 100 years trying to rationalize the structure-function relations of the phloem of plants. This tissue, ramifying throughout the organized plant body and extending within microns of the apical meristems, provides for the distribution of foods to all living cells. Evidence for a correlated movement of solutes and solvent throughout this tissue system has been strengthened by autoradiographic studies on whole intact plants. Evidence from the electron microscope indicates that the last serious barrier to acceptance of this mechanism - the nature of the protoplasmic strands that traverse the sieve plates - has been removed; Esau and her students have demonstrated that these strands, in the functioning condition, are tubular.

Interpretations presented throughout the volume and stressed in chapters III and XVI are derived not only from the autoradiographic studies but also from observations of plot work and physiological information gleaned from the literature. This is the result of the broad field of study undertaken at the University of California at Davis, and the constant attempt being made to integrate laboratory research and field studies. It is sincerely hoped that plant physiologists will find satisfaction in the use made of their science in the field of herbicide mechanism, that researchers in weed science will find elucidation of some perplexing problems of herbicide physiology, and that agriculturists will be aided and encouraged by the valuable information obtained from modern isotope techniques.

The writers wish to acknowledge with gratitude the help from colleagues and students, including Charles McCarthy, Barbara Kean, Douglas Steward, Otto Anderson, Mahmood Clor, Myra Mihajlovic, Chester Foy, Henry De Stigter, Fred Slife, Joe Key, Bernard Forde, Jose Pereira, George Mason, Richard Hull, and Fred Boyd.

We also appreciate the donation of labeled compounds by the following organizations: Am Chem Products, Inc., American Cyanamid Co., Chipman Chemical Co., Diamond Alkali Co., Dow Chemical Co., E. I. du Pont de Nemours and Co., Geigy Agricultural Chemicals, Naugatuck Chemical Division of the U. S. Rubber Co., Pineapple Research Institute, Rohm and Haas Co., Spencer Chemical Co., Stauffer Chemical Co.

Davis, California
December 19, 1963

SECTION A -- Methods

Chapter I. Introduction

Acquainting young people with some of the exciting discoveries that man has made in the exploration of his world is both a privilege and a challenge for the teacher. On several occasions it has been our pleasure to explain to young people what botany is and what botanists do - and always these young people are eager to learn more of the world of plants and of useful products of forest and range and cultivated field. The story of photosynthesis, the process by which plants capture the sun's energy and utilize it to produce chemical products essential to the whole biological world, is always thrilling to the scientifically inclined. A description of the processes of absorption and translocation by means of which plants are able to acquire minerals from their environment and utilize them to develop their own bodies is stimulating to students with aptitudes for chemistry or engineering. And, finally, to describe the history of early research on transpiration, the rise of sap in tall trees, and the movement of food in plants, is always to awaken interest in those who like mechanics.

In these days of higher specialization, it is well to remember that the food we eat and the clothes we wear are largely the result of the coordinated activities of whole plants. Therefore, research which seeks a complete picture of plant functions through study of the integrated activities of particulates, organelles, and grosser plant structures, is of the first importance. This volume describes discoveries that have filled many gaps in our knowledge of whole-plant function, and documents a new technique for the study of plants.

In all probability plants originated as single cells in the sea. The first cell involved a staggering number of structure-function relationships, and modern higher plants have all of these relationships plus countless more. It is through the development of diverse structures and their impact on plant function that many plants in the sea grew huge, that certain sea plants invaded the land, and that, on land, plants have been able to live in the tropical and the polar regions, in the osmotic concentration of saline lakes, and in montane environments.

Botanists were early intrigued by the great variety of form found in plants; later they delved into the functions of these myriad structures; now they pursue structure to its ultimate molecular state as they study the fundamental chemical and physical bases of function. In these studies one of the most useful of the newer tools is the radioactive isotope. By using isotopic labeling, innumerable natural and synthetic compounds may be converted to tracers that enable the biochemist and plant physiologist to explore areas beyond the reach of older methods.

The use of naturally-occuring radioactive isotopes in medicine is well known. The wider use of artificially-produced isotopes awaited their large-scale production in accelerators of various designs. By using neutrons to bombard elements, E. Fermi and his associates had contributed many new isotopes to science by the mid-1930's; by the end of 1935 about 100 artificial radioactive elements had been discovered. Since then, the development of the cyclotron has resulted in production of many radioactive isotopes, and by 1947 over 1,000 induced radioactive isotopes were known. The attainment of the self-sustaining nuclear chain reaction with uranium marked another milestone in

the field of artificial radioactivity.

Ruben and Kamen (1940, 1941) reported the discovery of carbon-14 in 1940-41, but this useful isotope was not generally available until an inexpensive method of production by the use of the uranium reactor was perfected. By 1947 the United States Atomic Energy Commission Price List No. 2 listed 74 radioactive isotopes and the price of a millicurie of carbon-14 as barium carbonate was down to $50.00.

Today there are a great number of radioactive isotopes available for research, and progress in their application in a multitude of uses has been rapid. In addition to carbon-14 with its extremely long half-life of about 5,000 years, sulfur-35, chlorine-36, phosphorus-32, calcium-45, and zinc-65 have proved very useful as tracers in plant physiological research. These materials have enabled scientists to make outstanding advances in such fields as photosynthesis, respiration, ion uptake and transport, translocation of organic nutrients, and in the synthesis and use of labeled compounds of physiological activity, including enzymes, co-factors, growth regulators, and inhibitors.

When used on plants dried in the frozen condition, autoradiography gives an accurate picture of the distribution of a given tracer in a plant that was intact at the time of treatment and subject to no more drastic treatment than the application of a droplet of solution to a leaf or stem, or inclusion of the tracer in the culture solution around the roots (Crafts and Yamaguchi, 1960*b*). So far as the authors have been able to determine, radioactive tracers in the quantities used have no deleterious effect upon the plant tissues because of their radioactivity. On the other hand, stimulators or inhibitors of growth, or physiological agents that cause chlorosis, inhibition of oxygen evolution, cessation of mitosis, or contact injury, seem to be unchanged in their activity by radioactivity of tracer elements included in their composition.

Here it should be emphasized that autoradiography is only one method for studying plants; for a complete record of plant behavior, counting, extraction, chromatography and other methods should be used. When long-lived tracer elements are used it is possible to autograph plants and then to carry out more accurate quantitative determinations upon them. When short-lived tracers are used, parallel treatments may be made so that counting or chromatography can be carried out while autoradiographs are being exposed. And, finally, where a detailed determination of the exact locus of uptake and transport is desired, histoautoradiography (autoradiography of sectioned materials) can be used to pinpoint the channels of transport and the final site of action of a labeled compound.

While the autograph of a treated plant gives a complete picture of the distribution of a mobile compound in the plant, quantitative interpretation of the picture obtained must be made with caution. As explained by Yamaguchi and Crafts (1958), the autographic image ranges from a light trace to an opaque black area. Foy (1958) found a close correlation of intensity with counts within the range of visible differences. Beyond the first opaque image, no further estimate of quantity of tracer can be made from a given autograph. By using different exposure times on the film, varying intensities of image may be had. Counting on the plant materials may also be used to determine radiation density. With most plant materials, incineration or extraction is not necessary; by construction of a standard self-absorption curve and standardization of technique, quite accurate counting can be done on ground plant materials (Foy, 1960).

The techniques used in plant autoradiography are relatively simple; they are easily taught, and equipment is relatively inexpensive. The screen trays, the vacuum chamber, the plant press and the materials used in the final exposure of the X-ray films are easily obtained and rather simply fabricated. The original steel vacuum tank (Yamaguchi and Crafts, 1958, fig. 14) has recently been supplemented by tanks of light metal, and the original simple wooden press has been replaced by a steel screw press. Additionally, the original sponge rubber separators used in the autographing bundle may be replaced with polyethylene sponge sheets, and the plywood separators with aluminum sheets.

Other such improvements will be explained later in this publication.

Results obtained by autoradiography are striking, and often exciting: evidence has been produced for hydrolysis of esters (Crafts, 1959*a*), for apoplastic and symplastic movement of assimilates and tracers (Crafts, 1961*b*), for general vs. localized distribution of compounds in plants (Yamaguchi and Crafts, 1958, fig. 10 vs. fig. 17), for a correlation of tracer movement with food movement, and for widely differing patterns of distribution of different tracer molecules. As new labeled compounds are synthesized their behavior in the plant can readily be studied, and in the field of pesticide research the role of translocation in systemic action can be rapidly determined. Thus new and valuable information is constantly being supplied by scientists working with isotopic labeled compounds. Indeed, use of labeled tracers in plants is proving to be one of the major advances in scientific technology.

The technique of interpreting plant autoradiographs has made rapid strides. Just as the physician studying X-rays may find evidence of injuries, abnormalities, malignancy, and disease, so the investigator studying plant autoradiographs has learned to recognize many significant signs of important physiological processes. The bypassing of mature leaves by tracers undergoing symplastic movement in the phloem, the intense labeling of buds, shoot tips, and root tips, the interinteraction of one compound upon movement of a second labeled compound, the relation of acropetal to basipetal movement in stems, the accumulation of some compounds and the fixing of others in nonliving tissues, and finally, the faint labeling of untreated leaves by compounds that characteristically migrate from phloem to xylem - all are examples of the types of response that lend themselves to such interpretations.

Other techniques are also relevant in plant studies: the evolution of $C^{14}O_2$ from tracer-treated plants, the determination of metabolic products of tracer denaturation by chromatography, and the use of $C^{14}O_2$ to study disturbed paths of metabolism in pesticide-treated plants, are some examples. Histoautoradiography is useful in studying the increase of various constituents in developing tissues and cells. We have found its application to problems of uptake, distribution, and metabolism very difficult. The point which we wish to emphasize here is the great usefulness of gross autoradiography in studying the initial penetration, the rapid translocation, and the ultimate distribution of various compounds in plants.

Chapter II. Methods of Autoradiography

PLANT MATERIALS

Almost any plant species that lends itself to greenhouse culture can be used for autoradiography. Where it was desirable to use small intact plants, the authors grew them from seed in nutrient culture. The seeds may be germinated in moist, washed sand or on cheese cloth suspended above a culture solution. As soon as the roots are long enough (usually 2-3 inches) the seedlings, held in holes in wide corks by cotton, are arranged in culture jars (fig. II,1) or, if details of the root systems are desired, are attached with masking tape to filter paper supported by glass plates (fig. II, 2). Barley, oats, corn, bean, cotton, soybean, nutgrass, sugar beet, tomato, Bermudagrass, rye grass, quackgrass, alfalfa, *Zebrina pendula* and *Tradescantia fluminensis* have proved convenient. Zebrina has hypostomatous leaves, so that cuticular vs. stomatal absorption can be readily studied; tradescantia comes in a number of variegated varieties and has been used to study translocation from green vs. chlorotic leaves (Crafts, 1961 *b*). Both are very easily propagated in the greenhouse, their growth can be readily regulated by the nutrient level provided, and they endure long periods of relative inactivity without loss of leaves when nutrients are depleted; they grow readily in soil, sand, or water culture.

Woody plants have been used both in the greenhouse and in the field (Clor, 1959; Leonard and Crafts, 1956; Yamaguchi and Crafts, 1959). Seedlings of oaks and coffee have been grown and treated in the greenhouse. Large shrubs and trees have been used in the field, and here careful handling of the labeled compounds is essential. (In working in state and national forests, permission should be obtained from the proper authorities before using isotopes).

In treating plants for autoradiography, thought should be given to convenient film sizes. The standard 10 x 12-inch X-ray film is very convenient and seedlings of many plants lend themselves to these proportions. When larger plants are needed, two 10 x 12-inch films can be placed end to end to accommodate a plant that is 24 inches long, or can be placed side to side to give an area 12 x 20 in-

Fig. II, 1. Solution cultures of barley, cotton, and soybean. Culture solutions have been reduced to a volume of 100 ml each in preparation for root treatment with a labeled tracer.

Fig. II, 2. Culture of Zebrina pendula roots on filter paper. Plants are grown to proper size (left). Transfer to paper is made by floating roots in water, inserting paper-covered glass sheet, tipping tray to leave roots on paper, fastening plant to glass-backed paper with masking tape (center), and inserting in darkened glass cylinder (right) which contains culture solution.

ches. A 14 x 17-inch film commonly used for chest X-rays is also available.

PLANT CULTURE

Convenience of culture is often a factor in the selection of plant species for experimental use. One-pint jars are very useful, and careful control of solution level, with the upper portion of the root system being exposed, may eliminate the need for forced aeration; such aeration is to be avoided in cultures having tracer applied via the culture solution, as the resultant splattering causes contamination. Plants which have proved useful here are red kidney bean, soybean, barley, cotton, corn, wild buckwheat, field bindweed, tradescantia, and zebrina; undoubtedly many other species will do as well.

In starting an experiment, two or more times the required number of seeds is used, and at the time of transfer to

culture jars rigorous selection is made in order to have uniform material. Careful examination for uniformity of vigor and development and equality of root growth reduces replication to a minimum. Where series experiments are performed using concentration, dosage, time, locus of treatment, or similar factors as variables, single plants are often used with perfectly satisfactory results.

In transferring plants from sand to culture jars, roots 2 or 3 inches long are preferable - if shorter, roots may be too small to be handled conveniently; if longer, they become intertwined and break when separated. Figure II, 3, shows bean cultures in the most convenient stage for establishment.

Plants may be treated at any stage of development. Bean plants *(Phaseolus vulgaris* var. *Red Kidney)* usually have the primary leaves fully expanded 11 to 14 days after planting; barley commonly requires 14 to 20 days to reach a proper stage for treatment. Under greenhouse conditions the culture solution may not require replenishing. If the treatment period is to be over 10 days the culture solution should be renewed just prior to treatment.

Nutrient-solution culture assures uniformity of plant growth, ease of harvest, freedom from root breakage and convenient control of inorganic nutrition. Hoagland's formula has been used in our work; barley grows well in full strength solution; beans and cotton do best in half-strength solution. Iron is added as Fe_2SO_4.

FILTER PAPER CULTURE

Filter paper culture is used to study details of root structure or accurate location of a tracer in roots following foliar application. Barley, zebrina, and rape plants have been employed, and others would probably be suitable. Plants for these cultures are started as usual, then transferred to culture solution and grown until the roots are 3 to 4 inches long. At the time of transfer to filter paper culture, filter paper of double or treble thickness is taped to long glass sheets that fit the culture jars. These culture plates are then partially immersed in a flat container of water, the

Fig. II, 3. Bean plants at proper stage for transfer from sand to water culture.

plant to be mounted is placed in the water, and the roots are spread in the proper position. The culture plate is then slowly raised from the water, leaving the roots adhering to the filter paper, and the base of the plant is affixed to the culture plate with masking tape. When the roots have been properly arranged, the culture plate carrying the plant is carefully lowered into its culture jar and enough culture solution added to come to within an inch or two of the root tips. The solution continues to moisten the filter paper by capillary action, and must be replenished to make up for loss from plant transpiration.

The culture jar should be covered with aluminum foil, and a sheet of aluminum foil (split so as to surround the plant stem) is used to close the jar on top. If the culture is to be maintained for an extended period, the filter paper should be sterilized in a 1 per cent sodium hypochlorite solution for 10 minutes and thoroughly rinsed; this will restrain bacterial and fungal growth.

TREATMENT

LABELED COMPOUNDS

Organic compounds labeled with C^{14}, S^{35}, or Cl^{36} are satisfactory for autoradiography. The inorganic isotopes P^{32}, Ca^{45}, Zn^{65}, Fe^{59}, As^{77}, and others, are also adaptable for autoradiographic use.[1] Although most manufacturers of labeled compounds provide materials of high purity, purity cannot be taken for granted; purity and specific activity may be assayed by paper chromatography, autoradiography of the chromatograms, and GM counting, direct or after extraction.

For ascending chromatography, 1 µl volumes of stock or treatment solutions are spotted 1 inch from the edge of Whatman #1 filter paper measuring 10 x 16 inches, and the paper is joined into a cylinder and placed in a glass vessel measuring 6 x 18 inches. A solvent system useful for several herbicidal compounds is isopropanol-NH_4Ac 20 per cent in water - glacial acetic acid, 79:20:1. Fifty to 100 ml of solvent are required and development time at 72 degrees Fahrenheit is 18 to 24 hours.

Counting is done on 1 µl samples of stock or treatment solutions slowly pipetted onto discs of lens paper in planchets. One-minute counts are usually adequate.

For most purposes, a specific activity of 0.5 to 1.0 mc/mmole is adequate for autoradiography. With highly toxic compounds, such as the chlorophenoxy acids, a higher activity ranging to 10.0 mc/mmole may be required if physiological injury is to be kept to a minimum and a satisfactory autograph obtained. Where a number of compounds are being used comparatively it is best to standardize the specific activity; here, the compound having the lowest activity determines the dosage. An alternative method for using compounds of differing specific activity is to vary the exposure time of the treated plants on the film, using an inverse relation between activity and time.

Many labeled compounds are available for autoradiographic use. Isotopes or labeled compounds, or both, are sold by several chemical companies, by a number of organizations specializing in work with isotopes, and by the United States Atomic Energy Commission at Oak Ridge, Tennessee. These materials are distributed in various quantities; common packaged quantities are millicurie lots and 50 microcurie quantities, although P^{32}, C^{14}-barium carbonate, and other materials may be obtained in much greater quantities. In plant autoradiography individual doses often range from 0.5 µc to 0.05 µc; these doses correspond to 0.5 µmole to 0.05 µmole of a material having 1 mc per mmole (a common specific activity). Figure II,4, gives the range of activity for C^{14}- containing compounds.

VOLUME

Application for many of the treatments reported herein is in the form of a 10 µl droplet. Volumes may vary, but if too great in a given application the solution may run and cover an undefined

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1 See GLOSSARY, page 136, for description of technical terms and trade names used in this publication.

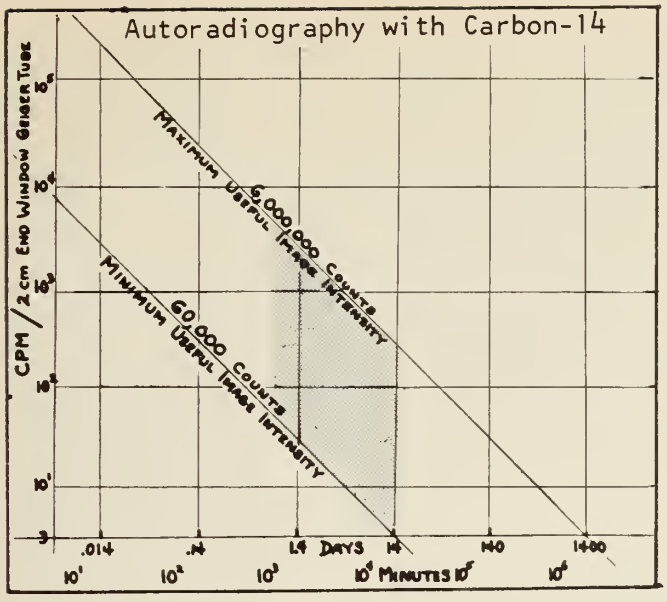


Fig. II, 4. Range of activity (shaded region) most useful for autoradiography with carbon-14. Range runs from 14-days exposure for a solution producing 3 counts per minute, to 1 day for a solution producing 4200 counts per minute. Activities outside this range may be used, but are inconvenient.

area. Treatments are usually placed within a lanolin ring about 0.5 cm in diameter; under warm weather conditions, the lanolin is thickened with dry starch or water so that it will not flow. Where a larger dose is desired, high specific-activity tracer may be used; another method is to apply several drops at intervals, allowing time for drying or absorption of droplets between applications.

STOCK SOLUTIONS

Because only a few micrograms of chemicals may be used in one experiment, it is convenient to store the original compounds in stock solutions involving, often, several milligrams of the compound, and to make up treatment solutions from these by dilution. Because some labeled compounds break down as a result of self-irradiation, our practice is to prepare them in stock solutions in acetone and to store the solutions in sealed ampules in a refrigerator (fig. II, 5). The compounds are weighed on a microbalance using micro-weighing dishes and a micro-spatula, or on an analytical balance having a special support to hold the container (fig. II, 6). To weigh, set the balance at approximately the desired amount, remove container top and carefully transfer tracer to the aluminum boat until the balance tips. Weigh the amount in the boat, dissolve in the proper volume of solvent and store in an ampule. From the stock solution, small aliquots may be taken with a micropipette to make up treatment solutions.

Some stock solutions may be made up in alcohol, some in acetone, some in water, etc., depending upon the solubility of the compounds. If not objectionable, 50 per cent alcohol is preferred over aqueous solutions because droplets of the alcoholic solvent do not adhere to the walls of the container, nor does this solvent support bacterial growth. If a stock solution is to be kept in a stoppered tube the stopper should be thoroughly coated with high-vacuum silicone grease to prevent loss of solvent. Stock solutions are usually made up to 50 μc per ml in quantities of 1 to 2 ml and labeled with the name of the chemical, its specific activity, its concentration in ppm, its weight and number of microcuries per unit volume, the name of the solvent, and the date; stock solutions are also often made up to solute concentration of 10^{-1}M, or 100 μmole/ml. Stock solutions are kept in a test-tube holder stored in a refrigerator.

TREATMENT SOLUTIONS

Two aspects of the treatment solution are important: the quantity of chemical in the treatment dose, and the intensity of the radiation in the treatment dose. Where penetration and translocation with a minimum of physiological response (growth regulation, toxicity) are being studied the tracer should be at the maximum specific activity obtainable. For example, if 2,4-D* (radioactive 2,4-D, MW 221) of a specific activity of 10 mc per mmole is used and the treatment dosage is to be 0.05 μc per treatment in 10 μl of solution, the treating solution should contain 1.105 μg per 10 μl or 110.5 μg per ml. Applied at a rate of 1.105 μg per treatment 2,4-D will cause a slight but perceptible formative effect on young cotton plants, leaf malformations may be detectable on bean seedlings, but barley, oat, and zebrina plants will show no effect. Such dosages are not injurious -

Fig. II, 5. Ampules of C^{14}-labeled phenylacetic acid (standing in block) and barium carbonate (right side of block) as received from distributor. Stock solutions in acetone are stored in ampules (in pint jar, right).

they do not inhibit uptake and distribution within 24 hours and plants will recover from them in time. If 2,4-D* having a specific activity of 1.0 mc per mmole is used, the same microcurie dose involving 11.05 µg per treatment will cause drastic formative changes in cotton, severe changes in bean, but no detectable changes in barley. Where only minor injury may be involved, if penetrability is low it may be necessary to increase dosage of the chemical. For example, a compound having the same molecular weight as 2,4-D and having a specific activity of 0.5 mc per mmole, 0.10 µc in 10 µl, will involve 44.2 µg - sufficient to bring about greater penetration and to cause drastic injury if the compound has the same toxicity as 2,4-D. If toxicity is low, there may be little or no injury.

Experience indicates that a dosage of 0.1 µmole per treatment in 10 µl of treatment solution usually assures adequate penetration. If the specific activity of the compound is lower than 1.0 mc per mmole, exposure time on the film may be lengthened to compensate for the lower activity.

When making comparative tests using labeled compounds the radioactivity of the various treatments and the chemical dosage should be uniform. Under these conditions, the researcher is limited by the lowest specific activity among the compounds being used. In studies involving a large number of labeled compounds we have used treatment solutions standardized at 0.5 mc per mmole; a specific activity of 1.0 mc per mmole gives more satisfactory results. In preparing treatment solutions from stock concentrates, unlabeled compounds of comparable purity are used, and these are used in making all tracer solutions to the common

Fig. II, 6. Support for tracer container used in weighing.

specific activity. It is most convenient to make two stock solutions, one of the labeled compound and one of the unlabeled; these are mixed to give the required specific activity and then diluted to treatment strength. Self-filling micropipettes are handy for preparing treatment solutions, and solutions of the desired volume can be made by careful calculation.

Using 2,4-D* as an example, and starting with a stock solution containing 1.105 mg per ml of the labeled material: if the treatment solution is to contain 0.05 µc per 10 µl with a specific activity of 0.5 (instead of 10.0 as in the previous case), to prepare 1 ml of treatment solution would require 100 µl of the stock solution plus 100 µl of a stock solution of unlabeled 2,4-D containing 19 times 1.105, or 20.995 mg per ml. After evaporation of the volatile solvent these aliquots, totaling 2,210 µg or 10 µmole of 2,4-D, are made up to 1 ml with the solvent used for the treatment solution. The authors usually use 50 per cent alcohol containing 0.1 per cent Tween 20, but the choice of formulating materials depends upon the labeled compounds being used and the specific requirements of the problem. Preparation of 1 ml of treatment solution in the above example is a matter of the requirements of the experiment; 10 µl of each stock solution could be made to 100 µl of treatment solution if desired. Recent work on surfactants (Jansen, *et al.*, 1961) indicates the need for more research upon these important formulating materials.

Occasionally, small treatment volumes are desired - as in a case where treatments were made to single leaves of bermudagrass. Here, the leaves were fastened in a horizontal position and 2 µl droplets of stock solution of several labeled herbicides were used. If surfactant is called for, droplets in 50 per cent alcohol may be applied and allowed to dry prior to application of the tracer solutions.

PREPARATION OF TREATMENT SOLUTIONS

In preparing treatment solutions, a clean working space covered with heavy wax-lined disposable paper is needed. Also needed are proper stock solutions, a box of facial tissue, aluminum foil for wrapping waste facial tissue, culture tubes 10 x 75 mm, a test-tube support block, silicone stopcock grease, 50-ml beakers, medicine droppers, a slender tipped pipette, a set of self-filling micropipettes, a 1/4-oz. capacity syringe bulb to fit the micropipettes, and a graduated 1/10-ml pipette fitted with a control made from a 2-ml syringe (having a barrel lubricated by lanolin) attached by a thick-walled rubber tube (fig. II, 7); the 1/4-oz. syringe bulb should be perforated on top with a red-hot needle. (By careful manipulation of the finger tip on the perforation one soon learns to fill and empty the pipette at will). The 1/10 ml pipette is used to transfer the stock solutions to the 10 x 75 mm

Fig. II, 7. Equipment for treating plants with radioactive tracers. Wooden block holds 10 x 75 mm test tubes, a 20 x 150 mm test tube of washing solvent, and a 1/4-oz. syringe bulb and pipette standing in a 20 x 150 mm tube of washing solvent. Self-filling micropipettes are in front; a 1/10-ml pipette with a 2-ml attached syringe is immediately behind them.

test tubes for dilution to treatment solution strength. Pipettes should be rapidly rinsed with 95 per cent alcohol and blown dry after each use; traces of phenoxyacetic acids are removed by flushing with hydrochloric acid. Facial tissue may be used to take up the rinsing alcohol; when a particular operation or treatment is finished, all such tissue should be wrapped in aluminum foil and placed in a proper container for disposal.

In all transfer and washing operations, care must be taken to avoid spillage, to avoid formation of bubbles at the pipette tip, and to apply all solutions and washing alcohol to the sides of the containers. The bursting of bubbles and dropping of solutions from pipettes causes splattering and is bound to result in contamination.

Sealed ampules should be wrapped with masking tape, scored with a file and opened with care, using pliers and rubber gloves (fig. II, 5). If acetone is used as a diluent for storing solutions of labeled compounds the ampule is opened to allow the acetone to vaporize, and the chemical is then dissolved in 95 per cent ethyl alcohol and transferred to the treatment solution container, using a slender-tipped pipette. With care, one may transfer the original solution plus washings and still use only one-half the volume of the final treatment solution; water is then added if the final solvent is to be 50 per cent alcohol. Formulation is done in this way because the chlorophenoxy compounds are slowly soluble in 50 per cent alcohol, but are rapidly soluble in 95 per cent alcohol. Surfactant, if used, should be made up as a stock solution and added before bringing the treatment solution to final volume.

Some compounds require special solvents. EPTC has been dissolved in methyl cellosolve as a carrier; this solvent is apparently of low phytotoxicity. For root treatment, EPTC has been dissolved in a mixture of equal volumes of Tween 20 and acetone and then dispersed in the culture solution.

The butoxyethanol ester of 2,4-D is not sufficiently soluble in 50 per cent alcohol; it has been handled in pure acetone as a solvent with the container's stopper (which is coated with silicone stopcock grease) taped in place. The treatment solution is kept in the refrigerator, and solvent lost during storage is replaced by bringing the level back to a predetermined mark on the container. Water and water-alcohol, acetone-water, and other mixtures and solvents have been used in preparing treatment solutions of some 25 labeled compounds. Volatile solvents such as pure acetone and viscous oils as solvents are to be avoided because they make it difficult to handle and clean the equipment.

TREATMENT METHODS

Selection of the treatment area is important. Leaves or stems may be treated by applying solution to a small area, to several areas, or by spray or multi-droplet application. Figure II, 8, left, shows single-droplet treatments on barley and bean, and, right, multiple-drop treatments on soybean and cotton; figure II, 9, shows treatment on the phloem tissue on the side of a tree trunk. For study of phloem translocation from leaves, a 10 µl droplet over the midrib close to the base of the leaf gives the best results; application to a leaf tip or on spots around the edges of a leaf is much less effective (Crafts, 1956*a*).

Treatments are best made on a sunny day in the greenhouse or field, or under illumination in control cabinets. If dark treatment is used to show lack of translocation when photosynthesis is lacking the starch and sugar content of the leaves should be determined, as some species evidently continue to export foods for several days after being placed in the dark; others cease movement within 24 hours and studies on coffee plants indicate that about 96 hours are required to deplete carbohydrate reserves in their leaves.

Temperatures should also be watched. Below 70 degrees Fahrenheit, absorption and translocation by beans may be slow, while that by barley is faster. Temperatures around 80 degrees Fahrenheit are more satisfactory for transport studies; above 90 degrees Fahrenheit evaporation of the treatment droplet is rapid, but repeated application of solvent, or enclosure of the plant in a high-humidity

Fig. II, 8. Single-droplet treatments on barley (left), bean (center); multiple-drop treatments on soybean and cotton plants (right) with lanolin rings and dams used to localize droplets.

chamber, will prolong treatment time.

For treatment with labeled compounds via the roots, plants are most easily handled by growing them in nutrient culture. Beans, barley, soybeans, and similar plants do well in one-quart Mason jars. The quantity of labeled tracer used to treat a leaf will usually suffice to treat roots if only 100 ml of culture solution is used at treatment time. Injury to roots should be avoided.

Small barley and oat seedlings have been successfully treated when their roots were contained in 4 ml of solution in 12 x 75 mm culture tubes. When held for treatment times of up to 8 days, 2 ml sufficed to keep the plants thrifty. Three-week-old barley plants starting to tiller were treated by holding the roots in 250 ml of culture solutions containing the labeled tracer. Root treatments of a variety of types have been carried out in volumes between these extremes.

Extensive replication is not usually required in plant autoradiography. Where the degree of difference is not very great in the independent variable of an experiment, four replicates are often used; where wider differences are involved three or two plants may be used, and where time series are used and trends can be followed only one plant per variable may be used. In all experiments control plants should be included, as an untreated plant frequently produces a faint image due to volatile substances given off during film exposure. (Such effects must be taken into consideration in interpreting the images produced by treated plants.)

Treatment time may vary from a minute to a month or more, depending upon the objective of the experiment. At approximately 80 degrees Fahrenheit in the greenhouse, 2,4-D* treatment of a bean plant on a sunny day may result in movement into the stem within one hour, and into the roots within 3 hours. Movement of MH* in barley may be into roots in 3 hours, and may continue for 24 hours or more; movement into zebrina may be slower but thorough if sufficient time is allowed. Twenty-four hours proved sufficient time for appreciable movement of several tracers in woody stems and young woody plants (Yamaguchi and Crafts, 1959).

Fig. II, 9. Treatments on phloem tissue of a tree trunk.

The effects of local accumulation are important in the use of labeled tracers. The pattern of distribution usually develops quite fully in 4 to 16 days. Some compounds remain mobile, however, and redistribute from mature to young growing-tissues for even longer periods. With 2,4-D, accumulation in parenchymatous tissues along the transport route may limit the extent of distribution; with amitrole, maleic hydrazide (MH), and dalapon, accumulation is transitory and redistribution in the symplast persists for many days.

Migration of a tracer from phloem to xylem and vice versa may be an important factor in determining the final distribution pattern. MH* and dalapon* are known to move laterally in stems; hence, when the primary leaf of a bean is treated and sufficient time is allowed, the opposite leaf will show an image. Potassium and phosphorus distribute this way in many plants, while 2,4-D*, amitrole* and calcium-45 do not show the same transfer pattern.

After plants have been treated by droplet application (figs. II, 8, 11), the treated area should be covered with masking tape (fig. II, 14). This may be done at any time after the droplet has dried but is usually done just prior to the start of the freeze-drying process. Covering of the treated spot prevents contamination during mounting of the plants and gives a permanent record of the locus of treatment. Finally, all plants should be clearly labeled with labels that can survive the freeze-drying process; small cardboard labels attached with string, or masking tape tags with code numbers in pencil are satisfactory.

PREPARATION FOR AUTOGRAPHY

Freeze-drying has proved essential in studies where labeled tracers are employed to study absorption and translocation (Crafts, 1956*a*; Pallas and Crafts, 1957; van der Zweep, 1961). Treated spots are cleansed with facial tissue to remove lanolin rings and, in the case of foliar applications, are covered with masking tape to prevent contamination when mounting the dried plants.

Following leaf and root treatments, roots are removed from the culture jars and rinsed under running water. (Contamination of the unexposed portions of the root systems with the culture solution must be avoided.) Roots are thoroughly rinsed with tap water, placed on sheets

Fig. II, 10. Apparatus for treating with a volatile labeled compound.

of facial tissue to drain, and plants are then placed in the drying tray. If plants have had root treatment via the culture solution, the roots must be wrapped individually in waxed paper or facial tissue to avoid contamination.

The screen drying-tray should be placed on a sheet of stiff aluminum foil bent up to a height of about 5 inches on all sides to hold in the CO_2 from the dry ice. Plants are arranged in the tray by alternating the position of tops and roots; as plants are added, pulverized dry ice is poured over them through a 1/2-inch mesh screen covering the tray (fig. II, 15). When full, the tray is placed in the precooled metal freeze-dry tank (figs. II, 12, 13). The tank is then closed, placed in a deep freeze set at -15 degrees Centigrade and then hooked up to a vacuum pump. Only enough dry ice to freeze the plants should be used, as excessive ice prolongs the freeze-drying process and may fracture leaves. After removing plants from the culture medium, processing should be done quickly to avoid wilting, which prolongs the drying process.

When this process was first used, calcium hydride lumps were placed in the bottom of the vacuum tank to hasten drying; the spent material had to be removed each day. We now find that a high capacity 1/2 horsepower vacuum pump, protected by two large-capacity vapor traps connected in parallel and a smaller one in series, extends drying time slightly but eliminates the need for the dessicant.

Figure II, 16, illustrates the inside arrangement of the deep-freeze box now in use by the authors. The temperature control is set to maintain -15 degrees Centigrade. Defrosting is accomplished during

Fig. II, 11. Droplet application to leaf of Bermudagrass. The stem is fastened with masking tape, the leaf taped into a horizontal position, a band of lanolin applied to prevent creeping of the solution, and a droplet of treating solution applied.

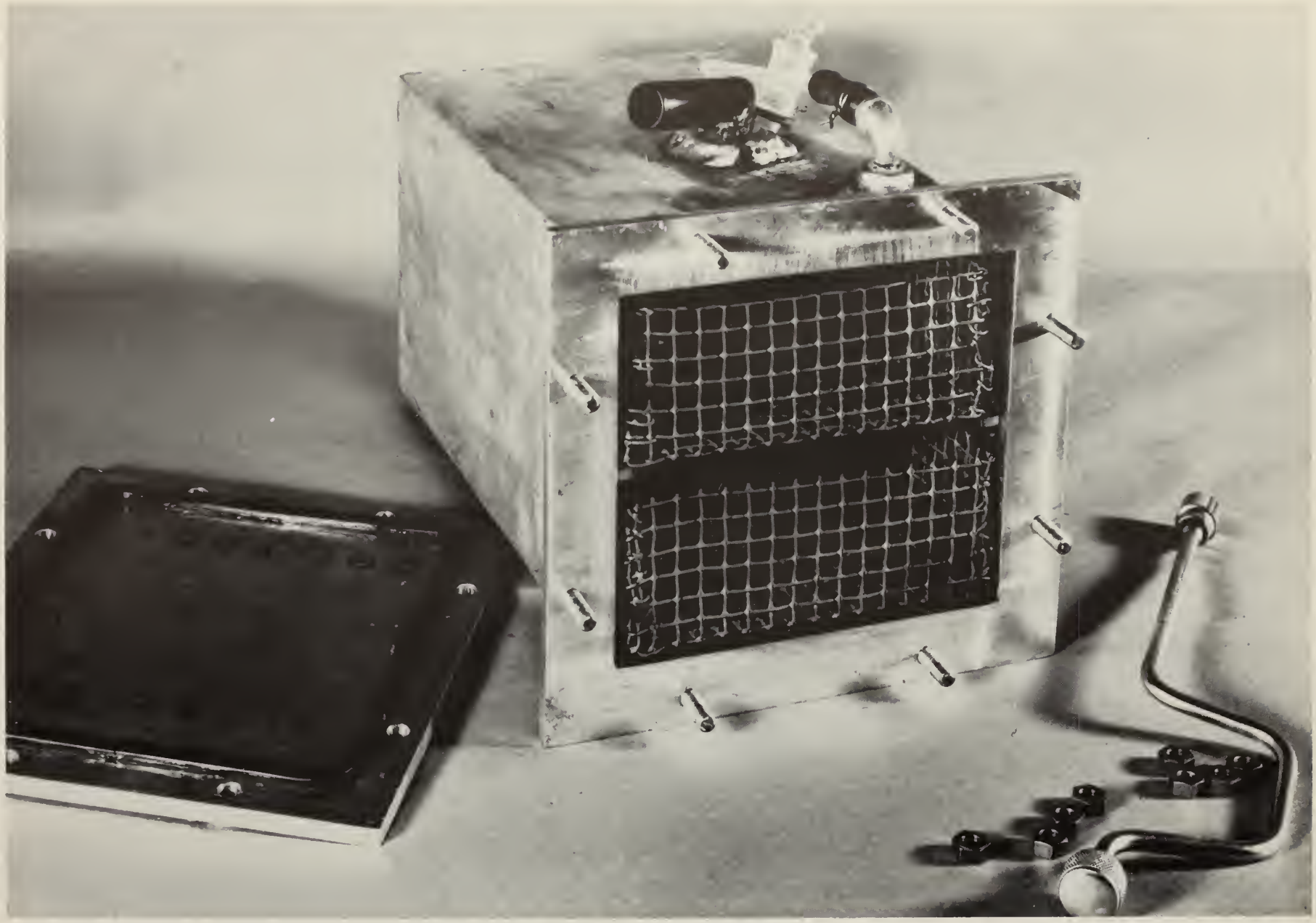


Fig. II, 12. Aluminum freeze-dry vacuum chamber with two empty screen trays. Cover (left) is fitted with a silicone-rubber gasket to hold vacuum.

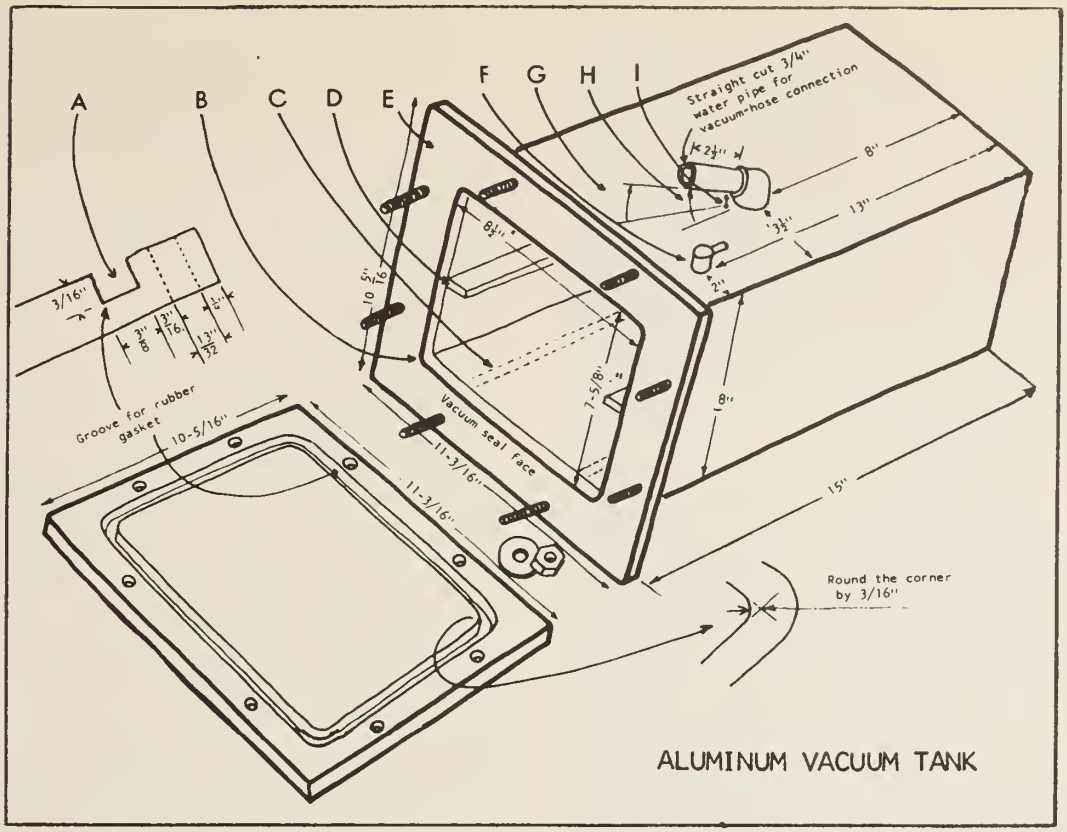


Fig. II, 13. Structural details of the aluminum freeze-dry vacuum chamber.

A. Gasket groove, 3/16-inch deep and 3/8-inch wide.
B. Corner of groove is rounded so that rubber gasket can be flared around the corner in the groove. Corner is rounded by 3/16-inch on the line of the hypotenuse; inside corners of the vacuum-seal plate are similarly rounded.
C. Skids on the bottom may be of aluminum (or other metal) 1/4 x 1/4 inch, and attached entire length of tank, about 1 inch from edge.
D. Runners inside tank are 1/8-inch thick and 3/8-inch wide; they are placed exactly in the middle of the side walls. Runners must support a tray weighing 2 to 5 pounds. End wall has no runners.
E. Vacuum-seal face is 1-11/32 inches wide; this allows an extra sealing surface of 1/8-inch on the inside of rubber gasket when lid is secured. Center of bolt shaft should be about 29/64-inch in from outer edge of the face.
F. Vacuum-releasing device. This consists of a 1/2-inch section of 1/8-inch water pipe welded over an 1/8-inch hole on a 3/4-inch section of 3/4-inch water pipe. The hole through the tank should be same diameter as inside diameter of 3/4-inch section so that hole can be used as housing for retaining something to soften the stream of air coming in from the vacuum release (vacuum-release valve is attached to the opening).
G. This arc should have an upward inclination of about 10 degrees.
H. This arc should be about 30 degrees.
I. Short arrow represents a distance of about 3/4-inch from top surface of tank to base of the "arm."

Outside overall dimensions of the tank are not given because they vary with the thickness of material selected. Total length from the back end to the vacuum-seal plate should not exceed 15 inches.

Fig. II, 14. Treated areas on barley and bean leaves covered with masking tape after droplets have been absorbed.

slack periods, as automatic defrosting would allow fluctuation in temperature and possible thawing of plants. If the door of the deep freeze has to be left open for extended periods while freeze-drying is in progress, blocks of dry ice applied to the vacuum tanks will hold them at the required freezing temperature.

Each vacuum tank is equipped with a vacuum-release mechanism consisting of a short pipe welded into the chamber, to which a 1/8-inch stopcock is attached with a piece of thick-walled pure gum rubber tubing (the stopcock should be lubricated with low-temperature silicone lubricant). Vacuum must be released slowly to avoid shattering the plant materials and to avoid too-rapid a temperature rise of the chamber and its contents.

One of the most difficult problems in the use of the vacuum tanks has been that of securing a tight seal between the lid and the tank. The lid has a routed groove (fig. II, 12, 13) into which is fitted a gasket of silicone rubber which retains its elasticity at -15 degrees Centigrade; if the groove is properly designed (fig. II, 13) the

Fig. II, 15. Plants (left) folded to fit the screen tray in which they are to be killed and freeze-dried. Center tray has received the pulverized dry ice; tray on the right is ready to place in the deep-freeze chamber. Aluminum foil retains the cold CO_2 atmosphere and assures even freezing.

gasket should stay in place indefinitely. Silicone grease is sometimes used on the smooth face of the tank to which the gasket is applied to lessen condensation and ice formation. If the stud-bolts that hold the lid in place are tapped into the face of the tank, a speed wrench may be used to secure the lid in place and to remove it when opening the tank.

The rubber tubing used in the vacuum line (fig. II, 16) has an inside diameter of 13/16-inch and walls 7/16-inch thick. It is soft and flexible at -15 degrees Centigrade and is slipped over the pipes, which are smooth but need not be tapered; the tubing is pure gum, has high elasticity, high tensile strength, and is long-lived. (A manifold to connect two or more tanks to the pump and pressure gauge was made of standard 3/4-inch water pipe fittings, smoothed to take the rubber tubing.)

The large vapor (H_2O) traps are designed to fit into one-gallon Dewar flasks, each having the capacity to trap about a pound of water. When in use the flasks are charged daily with small lumps of dry ice and covered with soft insulating material. About a pint of methanol or commercial acetone is poured over the lower one-third to one-half of the dry ice, causing a uniform condensation of moisture in the trap. Traps may be cleaned out each time the vacuum tanks are opened; the trap flask is held under run-

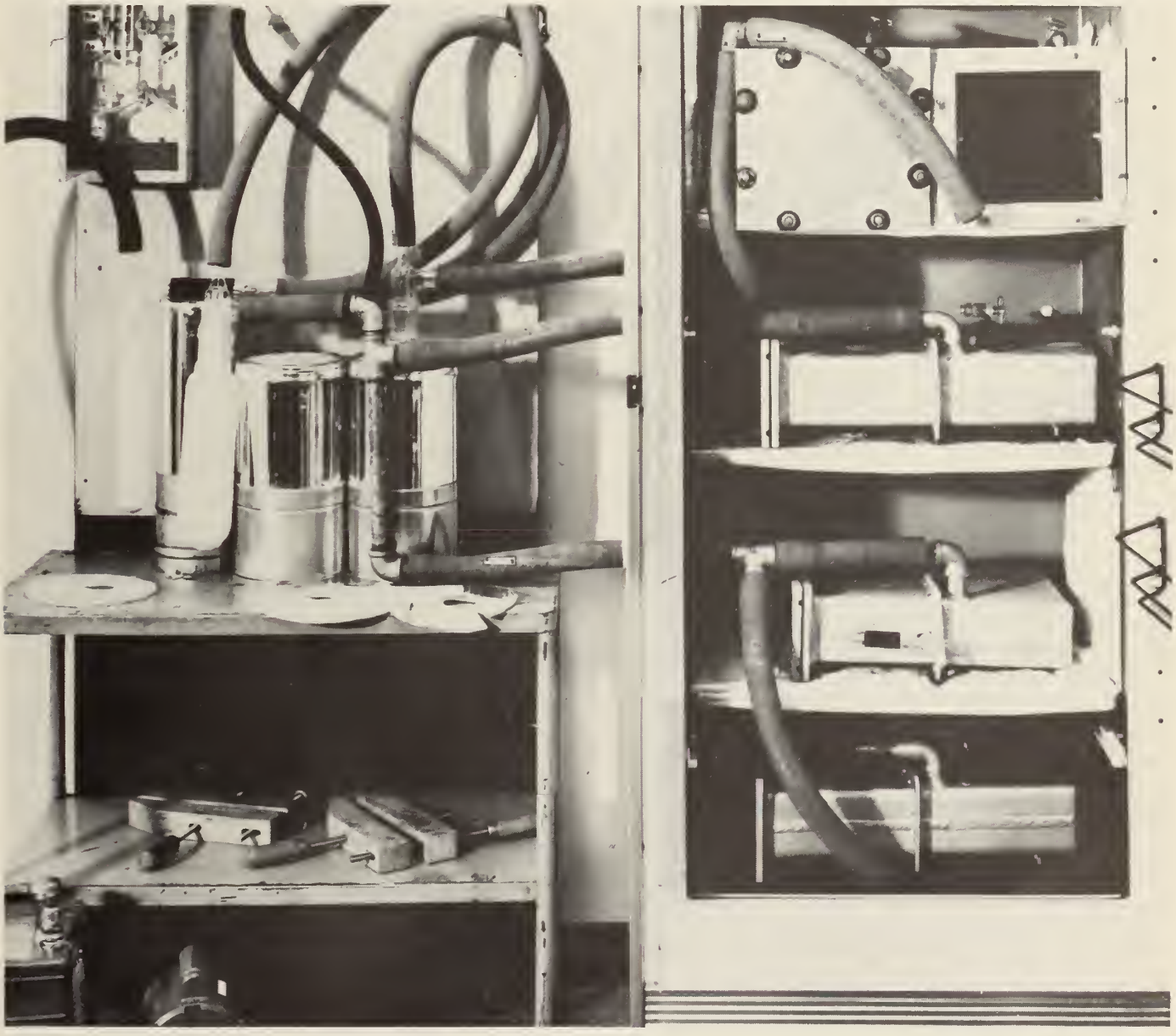


Fig. II, 16. Arrangement of freeze-dry chambers in the deep-freeze box, showing vacuum hoses, moisture traps, and vacuum pump.

ning water until the ice melts free enough to be taken out - if a trap flask is allowed to warm up gradually when loaded with ice the expanding ice will cause the flask to burst. Traps may be cleaned at any time without releasing the vacuum by clamping the rubber tube connections between the tanks and traps, thus releasing the vacuum in the traps, and melting the ice under running water (individual tanks may be isolated for servicing by placing clamps on the rubber vacuum tube). After replacing traps, clamps should be released slowly until the vacuum has been reestablished.

The third small trap is used as insurance against the possible loss of the dry ice in the larger traps. Because it traps little or no moisture during normal operation, the dry ice in this trap lasts longer and provides a safety trap.

If moisture passes all three traps and reaches the vacuum pump the oil will appear cloudy in the window; when this occurs the oil must be changed and the pump cleaned of all moisture before further use. These operations can be performed conveniently if traps are connected to freezer tanks and pump with sufficient lengths of flexible tubing.

Experience has proved that freeze-drying of plants is not successful unless the vacuum is below 2 mm of mercury. A range of 500 to 200 microns of vacuum has proved effective; the equipment pictured in figure II, 16 has reached 20 microns under freeze-drying conditions, and the pump now in use will bring the vacuum within this range in about 10 minutes. Use of silicone stopcock lubricant on the vacuum chamber lids and on all tube connections has helped in attaining such results.

Freeze-drying in a vacuum tank proceeds quite differently than does drying under unfrozen conditions. Thin leaves dry rather rapidly, but succulent stems and inner leaves of grasses dry more slowly and at different points: drying starts at the leaves, nodes and roots, and progresses along the stems. A partially freeze-dried plant may be completely dry in some regions and scarcely starting to dry in others.

Different plants require different drying times. Small cereal seedlings, young bean, and cotton seedlings and similar plants may dry completely in 7 to 10 days; large cereal plants, zebrina stems, and similar succulent tissues may require from 2 to 3 weeks. Storage of frozen plants in freezer compartments of refrigerators (approximately -12 degrees Centigrade) for 2 days, followed by normal freeze-drying, resulted in a longer drying time in the case of bean plants; similar treatment of cucumber plants had no effect. The nature of the drying of leafy plants suggests that water molecules escape via the stomata, and hence high vacuum is essential to rapid drying.

Bark samples from treatments of 24 hours or more have been dried in the open air. Samples of thin bark may be pressed flat between screens and dried in the open air, or in a forced-draft drying oven; a sheet of paper should separate the cambium side of the bark from the screen. With thick bark, it is best to pare away the outer part, leaving live inner tissue from 1/8-inch to 1/16-inch thick; this tissue may be dried between screens. Since the removal of the bark from the tree damages the translocation system and there is a slight movement of fluids, a slight artifact results (Leonard and Crafts, 1956; Yamaguchi and Crafts, 1959).

MOUNTING DRIED PLANTS

When the drying process is complete plant materials should be removed from the vacuum chamber, remoistened slightly (fig. II, 17) and mounted on white, sized paper, using casein or polyvinyl glue. Remoistening facilitates separation of plants that have dried in a single screen tray and helps avoid shattering the leaves, which are quite fragile when first removed from the vacuum chamber. Remoistening is most easily done in a closed chamber or covered pan with the plants resting in a screen tray which is open below to a standing-water surface. At 72 degrees Fahrenheit, barley plants are adequately humidified in 1 to 2 hours; large bean plants require 3 to 4 hours; and cotton plants may need 6 or 8 hours. Humidifying time can be shortened by using warm water, but condensation inside the humid chamber should be avoided.

Fig. II, 17. Screen trays holding freeze-dried plants are placed in enameled pans (left) with water below, and are then covered with sheet plastic. Remoistening helps keep plants from fracturing when handled.

When plants are sufficiently moist they should be removed from the moistening chamber, arranged on a sheet of white, sized paper, and glued down. For routine work the plant may be tacked down by gluing the main leaves and the roots and other organs (fig. II, 18); where mounted plants are to be used in demonstrations, all plant parts should be glued down (this is time consuming and not ordinarily done). The treated leaf should always be mounted with the treated side down, and information from the label on the plant should be copied on the lower edge of the mount. The mounted plants should not be dry enough to be brittle at the time of pressing. Pressing consists of covering the exposed side of the mounted plant with a piece of wax paper or aluminum foil, placing this covered face against a hard flat surface such as plywood or Masonite, backing the mount with several thicknesses of dry felt, and sandwiching a whole series so that all faces are against hard surfaces and all backs are against felt. A heavy (3/4-inch) piece of plywood is then placed on each side of the bundle and the whole placed in the press. Fig. II, 19, shows a simple wooden press; fig. II, 20, shows a press built from a screw-jack.

Fig. II, 18. A freeze-dried Tropaeolum majus plant placed for mounting.

Pressure should be great enough to compress the stems and completely flatten the face of the plants - a force of 50 or more pounds per square inch may be required; plants should be left in the press overnight or, preferably, for 24 hours (during which time the plant must dry thoroughly). After plants are removed from the press the thin cover sheets should be removed from the faces; these should peel off easily, but if there is trouble in removing them, Saran-wrap (or other plastic wrapping material) should be tried next time.

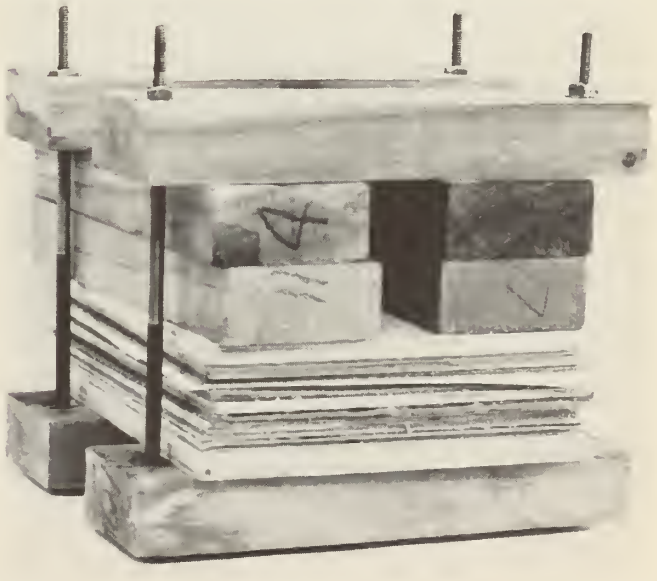


Fig. II, 19. Press made of 2" x 2" wooden bars.

On removal from the press the plant should be flat, smooth, and dry, and the thickness of stems, veins, etc. should be impressed into the mounting paper (fig. II, 21) due to the deformation of the felt backing. If sap (from incomplete drying) is squeezed from succulent stems, buds, or young grass leaves onto the paper, the contaminated areas should be carefully cut out and the mounting repaired. It is advisable to dry the plants so that this does not occur, but occasionally one may misjudge the drying; in such cases it is best to excise blotched areas, as they usually show in the final autograph.

AUTOGRAPHING

After removal from the press, the mounted plant materials may be allowed to stand in the laboratory to equilibrate with the atmosphere; they are then ready for exposure on the film. The authors use Kodak Royal Blue Medical X-ray film, a double-coated film either side of which may be placed against the mounted plant. In the dark room, the mounted plant is inserted with its face against the film, inside the folder in which the film is packed (fig. II, 22). It is advisable to pencil-mark each film to help in matching

Fig. II, 20. Screw-jack press. Aluminum sheets or plywood boards may be used as press plates; plant mounts should be backed with blotters to allow for the thickness of the stems and leaves.

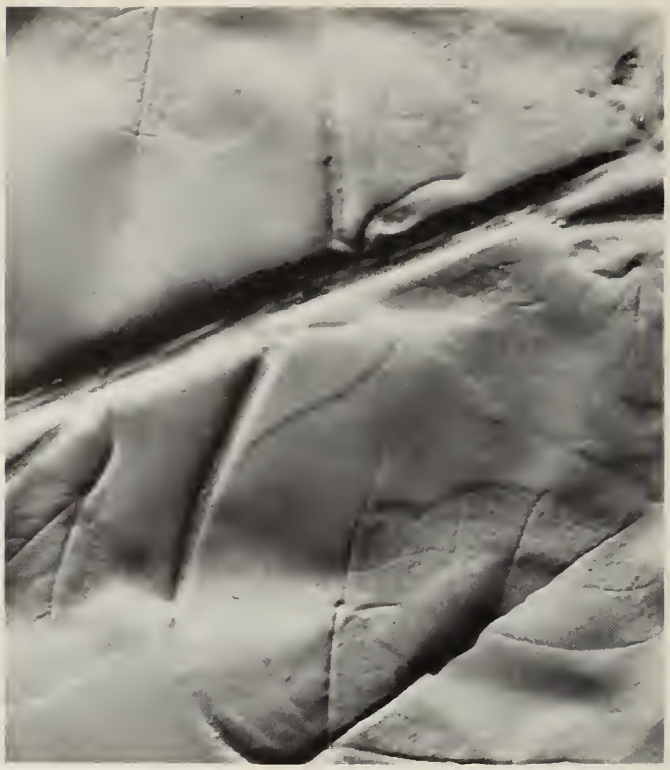


Fig. II, 21. Back view of a plant mount showing how backing with blotters allows the thickness of the plant material to be pressed into the mounting paper.

films and plants after the films are developed; in large experiments, radioactive ink can be used on the mounts. To prevent the control plant (without radioactive material) from producing a pseudoautograph from natural labile emanations, we have used a Saranwrap covering - this has kept out such emanations while permitting passage of 50 to 60 per cent of the beta particles.

Fig. II, 22. Inserting the mounted plant beneath the X-ray film in its folder. Exposure bundle of plants and films is shown on the right.

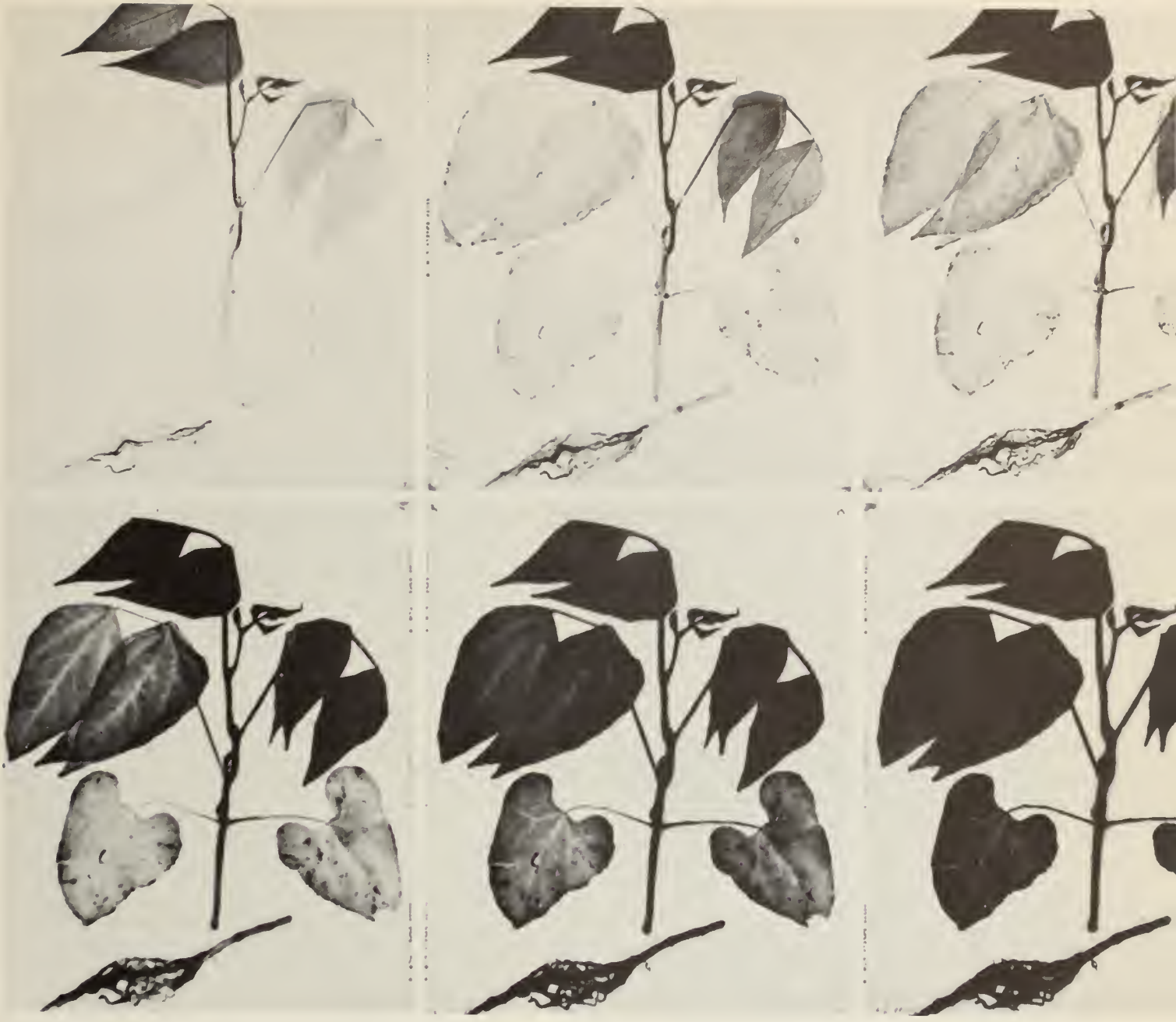


Fig. II, 23. Autoradiographs of a mounted plant having an unusually high content of C^{14}. Film exposure times were (top left to bottom right) 1/8, 1/4, 1/2, 1, 2 and 4 hours.

When all plants are so placed, the folders should be arranged with the film side against a separator of plywood with aluminum foil, or aluminum sheet of 1/32-inch thickness; this is to give the film a firm flat backing. With the folder on top of the separator film side down, a piece of 1/4-inch sponge rubber or polythene foam should be placed on the upper side to back the plant mount; the next folder should then be placed mount side down against the foam, and another separator put on top to back the film. The films and plants are sandwiched between separators and foam sheets until all plants are included after which stiff boards (1/4-inch plywood boards will do) are placed on both sides of the bundle and elastic belts are cinched around to hold plants and films firmly together; the bundle (fig. II, 22) is then placed in a light-tight box and left for the proper exposure time.

If plywood sheets are used for separators in the autoradiograph bundle they

should be covered with aluminum foil, as bare plywood may cause a pseudoautograph. A single thickness of aluminum-covered 1/4-inch plywood and 1/4-inch sponge rubber sheets provides adequate shielding between successive mounts when Ca^{45}, S^{35}, Cl^{36}, or C^{14} are being used; P^{32} and Zn^{65} require more shielding - such as several aluminum-covered separators and several 1/4-inch sponge rubber sheets. Equivalent stocks of newspapers will do for extra shielding.

Normal exposure of the plants on the film is 2 weeks, but exposure may be varied to bring out different features of the autograph. Figure II, 23, shows a bean plant that was exposed to a large amount of $C^{14}O_2$, allowed a time period of several hours before being freeze-dried, mounted and autographed. Exposures on the six films were as follows: top left to right bottom: 1/8, 1/4, 1/2, 1, 2, and 4 hours.

DEVELOPMENT

When exposure is complete, the bundle is opened, the films are removed, clipped in hangers and developed (following instructions in the Eastman Kodak Company's publication, *The Fundamentals of Radiography*). Optimum developing temperature is 68 degrees Fahrenheit, and development time is based on this; if this temperature cannot be maintained, development time must be changed accordingly, as explained in the above publication. We have used Kodak liquid X-ray developer and replenisher, and Kodak liquid fixer and replenisher, with complete satisfaction. Plastic and stainless steel developing, fixing, and washing tanks are available in sizes to fit the needs of the operation. An acetic acid stopbath is used between developer and fixer.

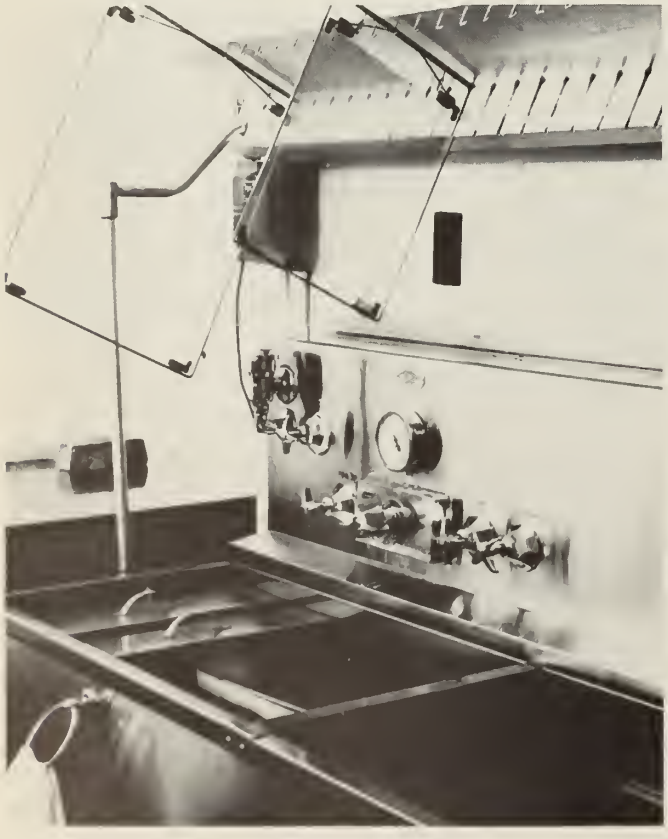


Fig. II, 24. Developing and fixing tanks, sink, and film rack for drying X-ray films.

The standard recommended development time for the Royal Blue X-ray film produces a dense gray background; we use only one-half this time as we want only a faint background for easy viewing - also, a dense background makes photographic reproduction difficult.

In removing films from the bundle in which they are exposed it is best to keep the plants and separators in the original order until the films have been processed - thus, if any stray marks or radio images appear they can be traced to their source. Sponge rubber sheets and separators occasionally become contaminated but such contamination can be readily detected if the development bundle is kept in order.

By careful planning one may save considerable time in the developing and fixing of films. We develop four at a time for 2.5 minutes. The films are placed carefully into the developing tank in order; after development, they are removed and dipped for 1 minute each in a stop-bath, then placed in the fixative and while there (for a period of approximately 10 minutes) four more are placed in the developer. Washing requires an hour, and drying usually requires 4 to 5 hours (fig. II, 24); after washing, each side of the film is wiped carefully with a wet sponge to remove dust or debris. When dry, each film is reinserted in its folder with the plant upon which it was exposed; for future use, it is advisable to put in a piece of the sized mounting paper at the same time. In photographing, this backing with shiny white paper will give the image maximum contrast. Material

from each experiment can be stored in a manila folder, and several experiments can be combined in each empty file box.

REPRODUCTION

In photographing autographs and mounts we use an easel as illustrated in figures II, 25, 26. The great contrast between the background of the autograph and the white backing of the mount presents a problem in reproduction. If the autograph and plant are to be photographed together, it is advisable to have a set of evenly exposed X-ray films, that have been developed, fixed and dried, to place over the plant mounts. The film used with each plant-autograph pair should be lighter than the background of the autograph - without such matching, different exposures must be used for plant mount and autograph or the latter will be much darker than the mount.

Fig. II, 25. Easel used in photographing plants and autoradiographs. Glass cover is raised for placing mounts and films in position. The chain and treadle enable operator to open or close the cover and to snap easel into position with the foot while holding cover with both hands.

The plants and autographs are placed on the padded surface of the easel (figs. II, 25, 26) and the glass front is closed to hold them in place. Reflected incident light from two or more photoflood lamps, placed on each side of the easel, gives satisfactory results. The easel used by the authors can be placed in a horizontal position while the plants and autographs are arranged; after clamping the covering glass in position the easel is rotated into a vertical position for photographing. A view camera is used for black and white photos, and a 35 mm camera is used for Kodachrome. By using a black curtain, with an aperture for the camera lens, glare on the glass of the easel can be avoided. Panatomic-X 4 x 5 inch film is used for making negatives, and Kodabromide paper is used for printing. Exposure is judged by a light-meter reading of the blank grey areas of the autoradiograph.

Fig. II, 26. Easel in position for photographing enclosed materials.

STORING OF PLANTS AND AUTORADIOGRAPHS

For future reference, the usual 10 x 12-inch mounts and films can be stored in an ordinary steel filing case. Each plant mount and its autographic film is kept in the film folder; the folders are grouped into manila folders, one for each experiment, and the folders labeled and stored in the filing case. The double-length films and mounts illustrated in figures X, 1 to 4 , are laid horizontally in a filing case drawer; the 14 x 17 inch films and mounts are stored in special filing cabinets made for storing X-rays in hospitals.

Freeze-dried plants require no special care in storage. We have plants stored for 10 years that look as well as they did when first stored; those in which C^{14}-labeled compounds were used still produce autoradiographs indistinguishable from the originals. During storage over several years many compounds sublime into the covering materials; when this occurs, the mounted plants may be re-autographed, and placed in new folders; new backing sheets for the films should be used. The discarded folders and paper should be handled as contaminated materials. These plants may be ground and counted to quantize the autoradiographs if desired. The plants may be extracted and chromatographed to study breakdown of the labeled compounds.

HARVESTING, MOUNTING AND DRYING OF DIFFICULT PLANT MATERIAL

Not all problems involving autoradiography can be handled by the routine greenhouse and laboratory methods described. In some cases, plants growing wild or in the field are required; these may include shrubs or full-grown trees. Any plants, herbaceous or woody, large or small, in the greenhouse, the field, or in a wild state, may be submitted to treatment with labeled compounds followed by autoradiographic analysis of absorption and translocation processes.

Large melon and cucumber plants have been grown in the greenhouse with their roots cultured on dark cloth over which the culture solution flowed slowly and constantly (de Stigter, 1961). These plants were freeze-dried, mounted (fig. II, 27), and autographed on 14 x 17-inch film, the foliar portion on one film (fig. II, 28) and the roots on another (fig. II, 29). The problem being studied involved compatibility of grafts, and radiographic analysis was critical in its solution.

With woody plants, cross-sections of stems and rings of bark removed from tree trunks have been used (Leonard and Crafts, 1956; Yamaguchi and Crafts, 1959). Such samples may be air-dried or oven-dried if the sampling techniques are designed to minimize artifacts - for example, in taking cross sections, the original cut should be at the base of the stem and subsequent cuts should be made toward the tip. In taking bark samples, simultaneous ringing in two positions with removal of the bark sample between cuts reduces the error of sap movement during the sampling process.

Stem tips and small tree seedlings (Clor, 1959) have been used effectively. The tree seedlings were grown and treated in the greenhouse; they were oven-dried, mounted and autographed whole. Care should be taken to avoid excessive pressure during the exposure of the mounted plant on film as deformation of the film will cause a pseudoautograph (fig. II,30). In work on grape vines in the field, samples have included leaves, stem sections, and stem tips; occasionally bark samples are taken at a time of year when bark will peel. In the case of large leaves such as grape, a section of the leaf involving the midrib and a centimeter or two of tissue on each side may be used; this section can be examined to distinguish between areas of accumulation - for example the veins, interveinal areas, or leaf margin.

In the case of the grape studies whole shoots were taken to the laboratory and sampled consistently from base to tip, taking cross sections of each internode and portions of each leaf. Samples were fastened to heavy filter paper with narrow strips of tape, freeze-dried, mounted (using casein glue), pressed, and autographed.

Stem sections from large woody plants, cut with a fine-toothed band saw, air-dried, sanded smooth, mounted and

Fig. II, 27. Mounted cucurbit plant ready to autoradiograph.

autographed have also given satisfactory results. (A vacuum dust-catcher should be used to avoid contamination from the dust, and a dust mask should be worn.) This work has been limited to plants treated with small doses of C^{14}-labeled compounds.

Large herbaceous plants are difficult to handle, but excellent results have been obtained with tobacco, cabbage and sugar beets. With the latter, sections approximately 2 mm thick were removed, placed on filter paper, frozen with dry ice, dried in the vacuum tank, mounted, pressed and

Fig. II, 28. Autoradiograph of plant in fig.

autographed. Both cross- and longitudinal sections have been prepared successfully.

Plants with complex, fragile leaves have been placed between sheets of light filter paper, rolled into open rolls, treated with pulverized dry ice and freeze-dried in the usual fashion (fig. II, 31). After remoistening, the freeze-dried plants are carefully unrolled, arranged on mounting paper and pasted down; thus, complicated leaf structures may be preserved through the freeze-drying process.

Fig. II, 29. Autoradiograph of the root system of plant in fig. II, 27.

Fig. II, 30. Pseudoautographs of the stems of a woody plant mount caused by excessive pressure in the exposure bundle. The only radioactivity in these samples was in the four blurred tips.

Fig. II, 31. A tray of freeze-dried tomato plants, and one plant spread out on the filter paper in which it was rolled.

HEALTH HAZARDS IN HANDLING LABELED COMPOUNDS

With care in handling, health hazards from using C^{14}, S^{35}, and Cl^{36} in the quantities used in autoradiography are negligible. Protective materials such as lead bricks, special aprons and film badges are not required, but rubber gloves and good hood facilities are needed. Radiation can be detected at the outer surface of the glass tubes in which the labeled materials are shipped, but can be eliminated by dilution when stock solutions are made up. Treatment solutions in bacterial culture tubes show no detectable radiation on the outer surface.

Because spillage may occur in transferring the labeled compounds during weighing, the area over which this is done should be protected with wax-surfaced paper or aluminum foil (distance of transfer may be minimized by the arrangement shown in figure II, 6). Transfer and weighing should be done in a closed weighing room, and all motions should be slow and careful.

Some labeled compounds are sticky, some are fine powders that adhere to all surfaces, and some are liquids. To facilitate handling, the total amount of the compound is dissolved in a given volume of solvent and made into a stock solution. Rubber gloves must be worn and work must be done under a hood. Portions of the stock solution may be transferred to small ampules and sealed, using a pipette with syringe control for such transfers. Stock solutions are kept in a refrigerator; those having volatile compounds or solvents are kept in sealed ampules, while others may be kept in test tubes with corks coated with silicone grease and fastened with masking tape or cellophane tape.

In handling the more hazardous materials, such as P^{32} or Zn^{65}, shielding should be used; rubber gloves and hood or glove boxes are necessary. Storing of such materials requires shielding; containers holding C^{14}-labeled compounds in quantities of several millicuries should be shielded. Film should be stored well away from radioactive chemicals; storage in a separate room is advisable.

Chapter III. Autoradiography: Interpretation of Results

Autoradiography is unique as a method for studying plants, as it allows the researcher to investigate processes occurring inside the intact plant. However, because the images obtained indicate distribution processes and chemical reactions inside living cells or within organized cell systems, interpretation of autoradiographic data is an important phase of the over-all method.

In the uptake and distribution of pesticides or tracers by plant foliage, a number of processes are involved. These processes include the penetration of the cuticular layers, either on leaf surfaces or within the stomatal chambers; they also involve the migration of ions or molecules from the epidermal wall to the chlorenchyma and conducting cells of the vascular systems. They require the passage of the molecules across the cell wall-cytoplasm interface that represents the external surface of the living plant body, and they include the movement of the molecules through living cells from epidermis to chlorenchyma to border parenchyma and into the sieve tubes of the phloem. Finally, they involve rapid transport from treated leaves and stems to remote regions of shoots and roots.

It has been proposed (Crafts, 1961*a*; Crafts and Foy, 1962) that in traversing the cuticle chemicals follow two routes: one lipoid, through which non-polar compounds move by diffusion, and one aqueous (made up of micropores) through which polar compounds may come into contact with the water continuum of the plant and move by diffusion or convection into the cell wall-cytoplasm interface. Inside the cuticle the moving molecules encounter the primary pectin-cellulose phase of the epidermal or chlorenchyma cell-wall system, and here they must move through a highly aqueous medium. Having traversed this medium, the molecules come in contact with the outer surface of the cell protoplast, a living membrane of lipo-protein composition having an energy supply enabling it to move and accumulate some molecules against a gradient.

Two terms introduced by Münch (1930) are extremely useful in considerations of the entry and translocation of molecules in plants. These are "symplast", the sum of the interconnected living protoplasm of the plant, and the "apoplast", the non-living cell-wall continuum that surrounds and contains the symplast. The concept of the symplast implies that all of the living cells of a higher plant are united into a continuum by means of protoplasmic connections, a fact now recognized by most plant scientists. Symplastic movment of substances is defined as movement confined to the living body of the plant. In contrast, apoplastic movement is movement via the interconnected non-living cell-wall and xylem phases. Symplastic movement is capable of carrying labeled sugar, derived from C^{14}-labeled urea, from the leaf where the urea is applied, to the utmost extremities of the shoot tip and of the root system and even into root hairs (figs. III, 1, 2).

It has been proposed (Crafts, 1961*b*) that the sieve tubes of the phloem are a

ramifving, integrated system of conduits, permeable to longitudinal movement and constituting a functional part of the symplast system. The tracheary elements of the xylem compose a specialized permeable system of conduits constituting a functional part of the apoplast complex. Evidence from autoradiography indicates that some tracer molecules enter and move in the symplast system with ease, while others move much less freely. The substituted urea and symmetrical triazine herbicides seem unable to enter and move in the phloem along with the assimilate stream. In contrast, most water-soluble compounds enter roots with ease but some, notably 2,4-D and benzoic acid, are moved to the foliar portion of the plant slowly and in small amount. These contrasting behaviors pose some extremely challenging problems both to plant physiologists (with respect to the mechanics of solute uptake and movement) and to agricultural technologists who wish to employ these mechanisms in using agricultural chemicals. The contrasting and unpredictable behaviors of many new chemicals pose a constant problem to those engaged in the use of pesticides and mineral nutrients, and this field is one in which plant autoradiography seems capable of making some outstanding contributions.

Early work with C^{14}-labeled 2,4-D, in which treated plants (killed by quick-freezing and dried between hot, dry blotters) gave indications of phenomenal and unprecedented rates of transport, was soon found to involve an artifact (Crafts, 1956*a*; Pallas and Crafts, 1957). The fact that the opposite leaf of treated bean plants receiving this treatment had labeling of the veins (fig. III, 3) led to the answer to this problem of transport. Theoretically, if 2,4-D* was being exported from one primary leaf with the assimilates it should not enter the opposite leaf via phloem. Critical studies

Fig. III, 1. Root of zebrina grown on filter paper, as shown in Fig. II, 2.
Fig. III, 2. Autoradiograph of root shown in fig. III, 1. This plant was leaf-treated with C^{14}-labeled urea; $C^{14}O_2$ released by hydrolysis has been converted to sucrose and moved in the transport system to the roots.

Fig. III, 3. Autoradiograph of bean plant treated on one leaf with a droplet of 2,4-D* then killed between blocks of dry ice and blotter-dried. Presence of the tracer in the opposite leaf proved that rapid movement of tracer was taking place during drying; this occurred in xylem because of evaporation from the leaf.

Fig. III, 4. Autoradiographs of bean plants treated on one leaf with 2,4-D* (left) and MH* (right), freeze-killed and freeze-dried. In this critical 3.5-hour treatment all movement was in the phloem; opposite leaves do not show.

proved this conclusion to be true (fig. III, 4); the label was moving in the transpiration stream and hence the mechanism was not truly symplastic. Thus, correct interpretation of data revealed by autoradiography led to the solution of this vexing problem (Pallas and Crafts, 1957).

In comparative studies with three herbicides, 2,4-D* moved a relatively short distance, amitrole* moved through the root system and accumulated in root and shoot tips where assimilate utilization was high, and mature untreated leaves were bypassed with no leakage from roots. In this test MH* moved freely throughout the symplast; a faint image could be seen on the filter paper on which the roots were growing, and all mature leaves showed light labeling. This was interpreted to mean that MH* was migrating from phloem of roots to the culture medium and to the xylem. Thus again, interpretation of autographs led to a realization of the importance of the concepts of symplastic and apoplastic movement, and to a recognition of the widely differing behaviors of different chemicals.

Plant autoradiography makes it possible to study a number of plant processes in intact plants not previously subject to critical investigation. A few of these processes are: (1) absorption of solutes; (2) translocation of solutes; (3) symplastic vs. apoplastic movement; (4) selective accumulation by tissue systems; (5) the relative mobility of contrasting molecular types; (6) the hydrolysis of esters in plant leaves; (7) the activity of urease and other enzymes in leaves; (8) the role of photosynthesis in food transport; (9) the effects of metabolic inhibitors on uptake and distribution of compounds;

(10) the localization of auxins, inhibitors and plant nutrients in the plant body.

Because in work on translocation by either the phloem system or the xylem conduits any injury of the plant may lead to erratic results, it seems apparent that such work must be done on intact plants. Before the introduction of isotopes, studies on phloem transport of viruses came nearest to meeting this situation. Now it is possible to study the penetration and translocation of labeled compounds without the injury caused by insect feeding or the risk of upset of metabolism and transport mechanisms by virus injury. Thus the use of autoradiography seems ideal for studies on absorption and translocation.

In working with physiologically active compounds it is important to know where a compound accumulates with respect to its true site of action. Autoradiography may be used to find the distribution of a labeled compound in the plant, to discover regions of accumulation, and to gain insight into its possible mode of action. Collection of $C^{14}O_2$ may give a clue to the breakdown of the compound, and extraction and chromatography may be used to discover evidence for chemical change in its composition.

By using compounds labeled in two or more positions, evidence of decomposition of a molecule inside plants may be readily obtained. By using labeled and unlabled forms of two compounds, interaction of one on the other can be investigated. Thus, synergism or antagonism between two components of a formulation may be studied not only in the test tube but inside the living plant.

Ashton, Zweig and Mason (1960) used $C^{14}O_2$ to study the effects of triazine compounds on CO_2 fixation by bean plants. Fixation was almost completely blocked at 1 ppm and above, and time studies at 1 ppm showed that three triazines all blocked $C^{14}O_2$ fixation in 6 hours whereas at 3 hours simazine inhibited, methoxysimazine had no net effect, and trietazine stimulated $C^{14}O_2$ fixation. In further studies Zweig and Ashton (1962) found that atrazine greatly modified the course of organic synthesis in bean leaves, and that sucrose synthesis was completely inhibited, whereas aspartic acid increased and glycine disappeared. Since citric acid, serine, and asparagine were affected, these workers concluded that atrazine was interfering with the tricarboxylic acid cycle and that respiration and dark CO_2 fixation were not being interfered with. Thus, by isotope treatment, chromatography and counting, basic studies can be made on plants intact during the treatment and reaction period.

Mason (1960) used tritiated 2,3,6-trichlorobenzoic acid (TBA) to study the uptake and movement of this herbicide. By autoradiography, he found a high concentration of label in shoot tips, and he proved by Warburg analysis that TBA stimulates respiration; he concluded that the high accumulation in meristems resulted from a sort of autocatalytic reaction: the herbicide moved freely to the meristem where it increased the rate of respiration, using up available substrate; this steepened the gradient of foods into the meristem and this in turn stimulated rapid transport which in turn increased the concentration of the herbicide, etc.

These examples indicate the type of problems that lend themselves to study by means of labeled tracers; in all such studies autoradiography is a major tool.

In the use of autoradiography certain experimental designs have proved useful:

1. Time series have proved essential in studies on absorption, translocation and redistribution. Initial penetration can be studied using short times, minutes and hours. Translocation via phloem or xylem starts only when the tracers have reached these vascular tissues; usually 1 to 3 hours are required; time series from this time to about 4 days will give a picture of the continuing process.

2. Redistribution following initial transport has been effectively illustrated by time series in barley and bean plants running 4 and 16 days. Even longer times can be used with tracers having long half-lives.

3. Concentration series have proved helpful in studying penetration rates. These can involve wide differences only

when compounds of high solubility are being studied.

4. Dosage series are excellent for studying the capacity of a transport system to distribute a tracer. Dosage series with 2,4-D* have been used to show the interrelation between concentration as related to uptake and total dosage as it brings about contact injury. In one 2,4-D* study with leaf application, a dosage of 88.4 µg or 0.4 mmole per treatment proved optimum in the series 0.02, 0.1, 0.2, 0.4 and 0.8 µmole.

5. Parallel comparisons are useful for studying the relative permeation and mobility of different compounds. In these trials many compounds are used on one or a few species.

6. Selectivity trials are useful in testing herbicides; in these, one compound is used on many species. In the latter two types of studies (4 and 5, above) many plants are grown under the same conditions, and treatments are made rapidly during a short time period so that time, dosage, and environmental variables are minimized.

7. Comparative trials, in which applications are made to the leaves of some plants and to the roots of others, dramatically show the great differences existing in the permeability of these organs, the selectivity of the phloem and xylem systems, and the role of migration and interchange between them in ultimate distribution.

8. Loci of application series on plants having many leaves have given strong confirming evidence that a mass-flow type of mechanism in phloem transport exists.

9. Treatment with tracers at different stages in the growth of plants has shown the division of labor among organs, the differing processes of synthesis, transport and storage of foods, the changing permeability of cuticle and root tissues, and the roles of maturity and senescence in plant function.

10. Uptake and distribution trials of extended duration have given valuable information on the locations, concentrations and decomposition of pesticide residues in plants.

SECTION B

Some Typical Problems and Their Solutions

Chapter IV. Mechanisms of Translocation

For almost 2000 years research has been done on transport mechanisms of plants. In the 17th and 18th centuries Marcellus Malpighi, the Italian anatomist, and Stephen Hales, the British plant physiologist, ringed plants in attempts to solve the riddle of sap movements, but it was not until 1894 that Dixon and Joly in Ireland elaborated a satisfactory theory to explain the rise of sap to the tops of tall trees. Their "cohesion theory", though attacked on all sides, has stood the test of time and is now generally accepted by most plant physiologists (Crafts, 1961*b*). Tracer research to be described in ensuing chapters has provided confirming evidence on the performance of this mechanism.

While the cohesion theory of water movement in plants accounts for a major portion of the transport accommodated by the apoplast system, the phenomena of active root absorption, root pressure, guttation, and xylem exudation require an additional mechanism. The root-pressure mechanism of Priestley (1920), as altered and elaborated by Crafts and Broyer (1938), seems adequate to cover the grosser aspects of active root uptake. The root-pressure mechanism, plus the cohesion mechanism of xylem transport, account for the major uptake and movement of water and inorganic salts in plants.

These mechanisms are described by Dixon (1914) and Crafts (1961*b*). Briefly, the root-pressure theory proposes that with the germination of the seed and growth of the radicle into the soil, salts are actively absorbed by the cortical cells of the expanding root, including the root hairs. As the ions of these salts enter the symplast system they are picked up by the streaming protoplasm and carried throughout the lumina of the cortex cells; they move by diffusion from cell to cell along the protoplasmic connections. Thus they traverse the cortex, cross the endodermis, pass through the pericycle and permeate the ground parenchyma of the stele.

The cells of the epidermis and cortex are well-aerated by the soil atmosphere and the elaborate intercellular space system of the cortex, hence they have ample opportunity to take up oxygen. But the cells of the stele are small, closely packed, and separated from the oxygen supply by the endodermis with its suberized Casparian strip in its radial walls. This strip constitutes an effective barrier between the cortex and the stele of the primary root, both for the influx of oxygen and the efflux of sap.

Accompanying this decreasing supply of oxygen between cortex and stele is an increasing amount of CO_2 liberated in the processes of respiration that provide energy for accumulation, protoplasmic streaming, cell division and cell differentiation. Thus, the cells of the cortex and the cells of the stele in the primary root find themselves in two different environments. The cortical tissue has an ample oxygen supply and a low CO_2 concentration; the stelar cells have just the opposite.

Concomitant with a decreasing oxygen

and an increasing CO_2 gradient from cortex to stele is an uptake and migration of salts that by diffusion and protoplasmic streaming are seeking an equilibrium of concentration. But, as the concentration in the stele approaches that in the cortex where active accumulation is taking place, the cells in this low O_2-high CO_2 environment are unable to hold the solutes, which escape from the symplast to the apoplast. With the gradient of solute concentration between the soil solution and the apoplast of the stele established by active accumulation in the cortex and release within the stele, water moves osmotically, volume increases, sap accumulates and - being unable to pass back across the Casparian strip - migrates into the xylem conduits and procedes upward in the plant. This process makes root pressure; it is responsible for xylem exudation from excised roots, for guttation from leaves, and for a form of apoplastic solute movement.

The above processes take place in young seedlings, in many herbaceous plants during the night, and in woody plants at the onset of root growth in the spring. As leaves expand and transpiration increases, however, the water balance in the apoplast system passes gradually to the negative side and water is moved (due to its cohesive force) in a state of tension. Thus, in most plants during summer days, in all plants growing in soils having a soil-moisture content approaching the permanent wilting percentage, and in all tall trees above the height of atmospheric rise (around 30 feet), water exists and moves in a subatmospheric or tensile state. While seeming to defy the common laws of the liquid state, this water is maintained in a metastable condition of super-heating by its internal pressure, its cohesion to itself and its adhesion to the thoroughly wet lignocellulose walls of the xylem capillaries, and also by the fact that within the apoplast system there are no unwet surfaces upon which vapor-phase may become initiated.

While the mechanisms described above seem completely adequate to account for water and salt movements within plants, the transport of foods is a different matter. Ever since the pioneering studies of Mason, Maskell and Phillis it seems well established that food movements in plants follow a source-sink pattern (Mason and Phillis, 1937). There has been long and heated controversy, however, on the details of the mechanism involved, one group (including Mason and his associates) maintaining that movement partakes of the nature of an activated diffusion, and a second group (exemplified by Münch) holding out for a mass-flow process.

It seems obvious that in most plants food substances are synthesized in the leaves and utilized in meristematic regions of the shoots and roots. Thus, the source-sink pattern of food distribution seems inherent in the physiological anatomy of the plant. With developing proof that the carbohydrates of plants move principally in the form of sucrose, at least during their transport via the phloem, it also seems obvious that within the plant there is a gradient of osmotically active solute paralleling the source-sink gradient. It is now generally accepted that the sieve tubes of the phloem constitute the common conduits through which rapid food distribution takes place, and it is known that the phloem and xylem are parallel systems within the vascular strands. Hence, with a ready and constant supply of water available in the xylem it seems obvious that the osmotic gradient inherent in the phloem system should result in a hydrostatic gradient between source and sink, and that, if the sieve tubes constitute a system of physiologically open conduits, a mass-flow of solution should occur in the phloem from source to sink.

What sort of evidence may be marshalled to substantiate this mass-flow mechanism in plants? The following points will serve to answer: (1) If one carefully cuts into the phloem there invariably occurs an exudation from this tissue which contains sugars and small amounts of nitrogenous nutrients. (2) Virus particles inoculated into the phloem by insects follow the same source-sink pattern of distribution as do foods. (3) If insects feeding on sieve tubes of the phloem (Mittler, 1957*a*, *b*; Zimmerman, 1961) are anaesthetized and cut free from their mouth parts, prolonged phloem-exudation occurs. This exudate contains the same compounds that are found in phloem-exudate

from cut stems. (4) When tracer molecules are applied to intact plants they may penetrate into the phloem and move with the assimilate stream. These tracers follow the same source-sink distribution pattern as food substances. They bypass mature leaves that are exporting foods, enter younger leaves that have not reached compensation, and concentrate in meristems of stems, roots, and shoots.

A good many experiments to be described in the following chapters throw new light on the relations of various cell functions to the translocation mechanisms, and several of them emphasize the complex interrelations between the symplast, the apoplast, the intercellular space system, and the conducting tissues that constitute specialized phases of these systems. Often overlooked in researches on vascular components of plants is the fact that these components constitute distribution systems adapted to the functions of total dissemination of solutes and not simply to movement from one locus to another. Viewed in this latter sense, it appears that the tracer studies included here provide a wealth of information on the distribution function in plants.

Chapter V. Evidences for Mass Flow in the Phloem

Autoradiography has been used for some 20 years to study uptake and distribution of labeled compounds in plants. Colwell (1942) supplied P^{32} to leaves of squash and used counting and autoradiography to study its location in various plant parts. Heavy labeling of the phloem groups of the bicollateral bundles indicated that P^{32}, when properly applied, moved out of the leaves via the phloem. Cross-sections of fruits indicated accumulation of the tracer in seeds, and P^{32} was also present in phloem exudate. Colwell concluded that movement of P^{32} in phloem paralleled movement of foods. Autoradiographic work on tomato by Arnon, Stout and Sipos (1940) showed accumulation of P^{32} in the seeds of this plant.

These early studies indicated the possible use of autoradiography for detailed investigation of the absorption and translocation of pesticides. Day (1952) showed that 2,4-D applied to the leaf of bean brought about bending of the epicotyl, and by careful studies he arrived at linear rates of presumed transport of this regulator. With the production of C^{14}-labeled 2,4-D it became obvious that a tool was available for use in critical studies on the absorption and translocation of this important compound by intact plants.

To attack this problem we posed two questions: Does 2,4-D as such get into a plant? If 2,4-D is absorbed, is it translocated into the regions of growth response unchanged?

Our first tracer experiment involved the use of carboxy-labeled 2,4-D-C^{14}, and provided for a 4-hour treatment time and two methods of killing the treated plants: quick-freezing between blocks of dry ice followed by drying between hot dry blotters, and killing between hot dry blotters by the method commonly used with herbarium specimens. Several plants were handled by each method and they were used to test exposure times of the dried plants on Kodak No-screen X-ray safety films. The experiment indicated the 4-week exposure period to be best, and proved that in the 4-hour treatment period 2,4-D* penetrated the leaf and was translocated throughout the stem and well into the roots. It proved also that the quick killing caused an artifact - the uptake and rapid transport of the labeled compound via the xylem to regions where drying was taking place (figs. III, 3, 4).

The artifact introduced by the quick-killing treatment is reminiscent of the early work with the acid-arsenical method for killing weeds (Crafts, 1933). In this method, an acid solution of arsenic is applied as a spray to perennial weeds; with the rapid killing of the mesophyll tissues of the leaves, the leaf-sap, plus arsenic which had diffused into it, is rapidly sucked back through the stem into the roots. This is typical apoplastic movement - it is the phenomenon that takes place during drying when tissues of the treated plant are killed by quick freezing and then allowed to thaw out; the solution moves along hydrostatic gradients to regions where drying is taking place (Pallas and Crafts, 1957).

Our next experiment involved a time series in which treatment times were as follows: 1/4, 1/2, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 36, 48, 72, 96, 120, and 144

hours. The quick-killing method was used. The plant receiving the 1/4-hour treatment had tracer in its opposite leaf, the 1/2-hour treatment plant had tracer in its opposite leaf and roots, and the 3-hour treatment plant was bent and had tracer in its opposite leaf and roots. Day (1952) had found that absorption through the cuticle and movement into the epicotyl takes somewhat over an hour; presence of 2,4-D* in the roots and opposite leaf of the plant treated for 1/2-hour indicated again the artifact of xylem movement. However, since the tracer was present in the stems and roots of five replicates in the 3-hour test and four of the plants showed the bean bend in the epicotyls, it seems evident that our first questions had received a positive answer: 2,4-D* was present in the bent epicotyl, the hypocotyl and the roots of all plants treated 5 hours or more. Movement into terminal buds, which could be possible only via phloem, required 6 hours or more.

Experiments of the type described were continued, using bean, cotton, and cucumber seedlings (Crafts, 1956*a*). Effects of water balance, droplet placement, leaf location and various methods of killing showed definitely that quick killing followed by rapid drying between blotters results in the artifact of xylem transport. Drying alone between hot blotters was slower, but the plants seemed to show a true picture of phloem transport. The greatest problem in this method is the lack of a definite treatment time, for translocation undoubtedly continues for some time after the plants are put in the press. On the other hand, freeze-drying in a vacuum chamber proved effective (fig. III, 4) and after further trials this method has been adopted for all work with small plants. From these early trials a few definite conclusions stand out:

(1) Following application in solution as a droplet, 2,4-D* penetrates the cuticle, migrates through the mesophyll, enters the phloem tissue and moves from treated leaf to growing roots and young shoots.

(2) Under some conditions 2,4-D* will move from leaf to roots; under other conditions from leaf to shoot tip; under certain conditions to both roots and shoot tips.

(3) Whenever the bean epicotyl bends as a result of 2,4-D* treatment the compound has penetrated the leaf, petiole and stem, and is present in epicotyl and hypocotyl.

Although our original conclusions were drawn on the basis of the presence of the tracer in the affected regions only, more recent work by bio-assay and chromatography has substantiated our view that 2,4-D attains this distribution.

TRACER DISTRIBUTION

Meanwhile, tests using 2,4-D* on seedlings of a number of plants were posing new problems. For example, when seedlings of field bindweed (*Convolvulus arvensis*) were treated on one cotyledon, 2,4-D* moved down into the roots without labeling the opposite cotyledon in some plants; in others it moved both downward into roots and upward into the shoot tip, while the opposite cotyledon was free of label (Crafts, 1956*b*). When tracer was applied farther up the stem on seedlings having several leaves, translocation still carried the tracer to the roots and mature leaves along the stem were bypassed. On the other hand, when a young growing leaf at the tip of the plant was treated the tracer did not move downward from the area of treatment but did move to the tip of the leaf (Crafts, 1956*b*, figs. 32, 33, 34, 35).

For over a hundred years there has been controversy as to the mechanism of movement of foods in plants. Two divergent views are: (1) translocation in the phloem system is a diffusion-like process in which each molecular species moves along its own independent gradient unaffected by movement of other substances, including solvent water; and (2) translocation in the sieve-tube system is a pressure or mass-flow in which food molecules, tracers, or any other molecules present in the conduits move together with the solvent water from the source of synthesis of osmotically active solutes to the sink, or sinks, where assimilates are utilized in growth, respiration and storage.

By a diffusion-like movement, tracer applied to a leaf should presumably move out through the petiole, and then traverse the stem to enter the entire plant.

The fact that 2,4-D* moving out from a newly treated leaf might move solely to the root, or to shoot and root, and that it consistently bypasses mature leaves that are themselves exporting foods, but enters young growing leaves that are consuming foods, indicates that this tracer moves in a stream with assimilates. Its failure to move out of young leaves that are importing foods implies the same sort of mechanism. Because this indicates the existence of a flow-type of mechanism, further confirmation of such a type of mechanism seemed desirable.

THE SOURCE

If mass-flow requires an active source of synthates, there should be a correlation between translocation and food synthesis. Earlier work with cold 2,4-D on bean had shown that this compound would not move out of a leaf starved by darkening until all food reserves were exhausted (Mitchell and Brown, 1946; Rohrbaugh and Rice, 1949; Weintraub and Brown, 1950). Addition of sugar to the 2,4-D applied under these conditions induced movement as did illumination.

Treatment of chlorotic leaves of variegated *Tradescantia fluminensis* plants in the greenhouse with 2,4-D*, amitrole*, and MH* resulted in no movement, while treatment on green leaves resulted in extensive movement. The latter two compounds are more freely mobile than 2,4-D* (figs. V, 1, 2, 3).

THE SINK

Having established the requirement for photosynthesis or other supply of food at the source, sink activity was next examined. 2,4-D* movement from the treated leaf of a plant that had been growing for 30 days in tap water was negligible; the root was long but it had ceased to grow and the plant had low activity. Treatment of a similar plant recently removed from a nutrient solution showed movement of tracer into roots; a

Fig. V, 1. Movement of 2,4-D* from green leaves (left) and a chlorotic leaf (right) of variegated Tradescantia fluminensis. Photosynthesis activates the assimilate stream which carries the tracer out of the green leaves; there is no movement from the chlorotic leaf.

Fig. V, 2. Movement of amitrole* from green leaves (left), and a chlorotic leaf (right) of variegated tradescantia.

plant still actively growing in a 1/2-Hoagland nutrient solution showed strong labeling of roots (Crafts, 1961*b*, fig. 8-5). Obviously, the more active the growth process in the sink, the more tracer moves into it.

To further study direction of movement from treated leaves of zebrina, several plants growing in a 1/2-Hoagland solution were pruned to leave three healthy leaves at the base and two to four young growing leaves at the tip. The two uppermost of the lower leaves were treated with a high specific activity 2,4-D* (6 mc per mmole totaling 1.1 μc per plant). Plants so treated for 9 days translocated tracer throughout their roots as well as into their tip leaves (Crafts and Yamaguchi, 1958, fig. 8).

The location of source and sink were also reversed in order to demonstrate that tracer movement is polarized not with respect to the organization of the plant but with respect to source and sink. Tall zebrina plants that had been growing vigorously in a 1/2-Hoagland solution for 1 month were pruned, removing the growing stem-tip and leaving two fully expanded leaves at the upper extremity of the stem. The two tip leaves were treated, using 1.1 μc of 2,4-D* with a treatment time of 9 days; here, movement took place through the total length of stem in a basipetal direction with ample labeling in the roots and in basal shoots. Again there was a two-way movement at the base of the plants, the assimilate stream dividing and carrying the tracer into two active sinks (Crafts and Yamaguchi, 1958, fig. 9). It seems obvious from these experiments that translocation proceeds from a source of assimilates to a sink, or sinks, where assimilates are being utilized in growth; meanwhile, other sources such as the lower leaves (fig. X, 1) are bypassed. (For further observations of similar response see Jackson, 1962.) Such distribution cannot be explained on the basis of a diffusion type of movement, and in 11 years we have failed to find an exception to this type of tracer distribution in plants. In cases where leakage occurs into the transpiration stream, leakage also takes place into the external medium of the roots.

Fig. V, 3. Movement of maleic hydrazide* from green leaf (left) and a chlorotic leaf (right) of variegated tradescantia.

ROLE OF LEAF LOCATION IN TRACER MOVEMENT

The mass-flow concept of phloem transport in plants requires that foods manufactured in leaves low on the axis should move into roots, that foods farther up might move both downward to roots and upward to sinks in young leaves, buds and the shoot tip, that those even farther up should move exclusively upward, and that young rapidly expanding leaves should be importing rather than exporting foods. If tracers are moved in this same stream they should undergo the same general distribution, save that they should be subject to accumulation in varying degrees according to their molecular properties. A common expression of this mechanism is the bypassing of mature leaves by tracers in movement from active sources to active sinks. Here we know that the phenoxyacetic acids and the benzoic acids are strongly retained in living cells, that amitrole is intermediate, and that maleic

Fig. V, 4. Results of treatment with 2,4-D* on one cotyledon of Polygonum convolvulus (left), leaf 2 (center), leaf 4 (right).

Fig. V, 5. Results of treatment with 2,4-D* on leaf 6 of P. convolvulus (left), leaf 8 (center), leaf 10 (right).

hydrazide and dalapon are freely mobile and may migrate from phloem to xylem. The author's early work (Crafts, 1956*b*) with 2,4-D* in field bindweed *(Convolvulus arvensis)* indicated that the above pattern of distribution was to be expected, and subsequent studies with zebrina plants gave many indications of the same pattern, with bypassing of mature leaves being a common phenomenon. In order to document this concept more completely, two experiments on the relation of leaf location to tracer movement have been conducted.

The first experiment involved a series in which *Polygonum convolvulus* plants growing in Hoagland's solution were treated with 1/10 μmole of 2,4-D* of specific activity 1.24 mc per mmole on various leaves and given a 1-day treatment. These plants had been growing in the greenhouse, had passed through the rosette stage, and were making rapid length-growth, but roots were apparently not making rapid growth since 2,4-D* did not move into them in more than trace amounts.

From the cotyledon (fig. V, 4, left) which was green and healthy, 2,4-D* moved principally downward but was present in the roots at a distance of 1 inch or more only in trace amounts. From leaf 1, 2,4-D* moved downward in the stem and upper root system; upward, it entered two shoots in the axils of leaves 2 and 3, and in small quantity it traversed the total length of the shoot to the tip. From leaf 2, (fig. V, 4, center), 2,4-D* moved into the shoot in the axil of leaf 2; it moved downward in the main stem into the upper roots, bypassing leaf 1, and in lower concentration ascended the stem to the tip, labeling two expanding leaves and bypassing three full-grown leaves. From leaf 3 downward, movement was still strong into the upper roots; at intermediate concentration, 2,4-D* moved along the upper stem to its tip, labeling two expanding leaves and bypassing four fully expanded ones.

From leaf 4 (fig. V, 4, right), the tracer went in both upward and downward directions in approximately equal concentrations; it reached the roots and traversed the shoot to its tip, labeling three expanding leaves, slightly marking a fourth, and bypassing three mature ones. Uptake by the fifth leaf was strong and 2,4-D* moved downward past four mature leaves into the roots; it moved in equal concentration up the stem to its tip, labeling one expanding leaf and bypassing two. From leaf 6 (fig. V, 5, left), 2,4-D* movement was predominantly upward; downward labeling faded within 2 inches, and upward there was strong labeling to the stem tip; one maturing leaf was almost completely bypassed, and a young one was strongly labeled. From leaf 7, movement downward was about 1 inch, and upward it was dense to the shoot tip; one mature leaf was bypassed and one young one had medium labeling. From leaf 8 (fig. V, 5, center), movement was upward; leaf 9, which was not fully grown, was lightly labeled. From the tenth leaf on one plant (fig. V, 5, right) and the eleventh on another, labeling from the point of application to the tip of the leaf was very dense; there was some movement basipetally along the midvein but no tracer left the treated leaf - in both cases the treated leaves were not completely expanded and were very evidently still importing assimilates. In all but the two uppermost leaf treatments, tests were run in triplicate with remarkable uniformity, and shorter series in which applications were made to stems at various levels gave the same general pattern of 2,4-D* distribution. Had amitrole, a more freely mobile tracer, been used there would probably have been more labeling of the roots.

The second experiment in this series was on nasturtium plants; amitrole*, 1/10 μmole at 0.94 specific activity was used in 10.0 ul droplets with 1/10 per cent Tween 20 in the formulations, and treatment time was 4 days. Nasturtium discards its cotyledons upon germination; in our plants the epicotyl averaged about 4 inches and there were a few roots at its base. The first two leaves come out as a pair from a single node, but above these the leaves alternate. From one of the first pair of leaves (fig. V, 6, left) amitrole* moved predominantly downward; roots were strongly labeled whereas labeling in the tops was faint to medium among the three replicates. From leaf 4 (fig. V, 6, right), movement was about equal judging from the density of stem labeling. In this plant, amitrole*

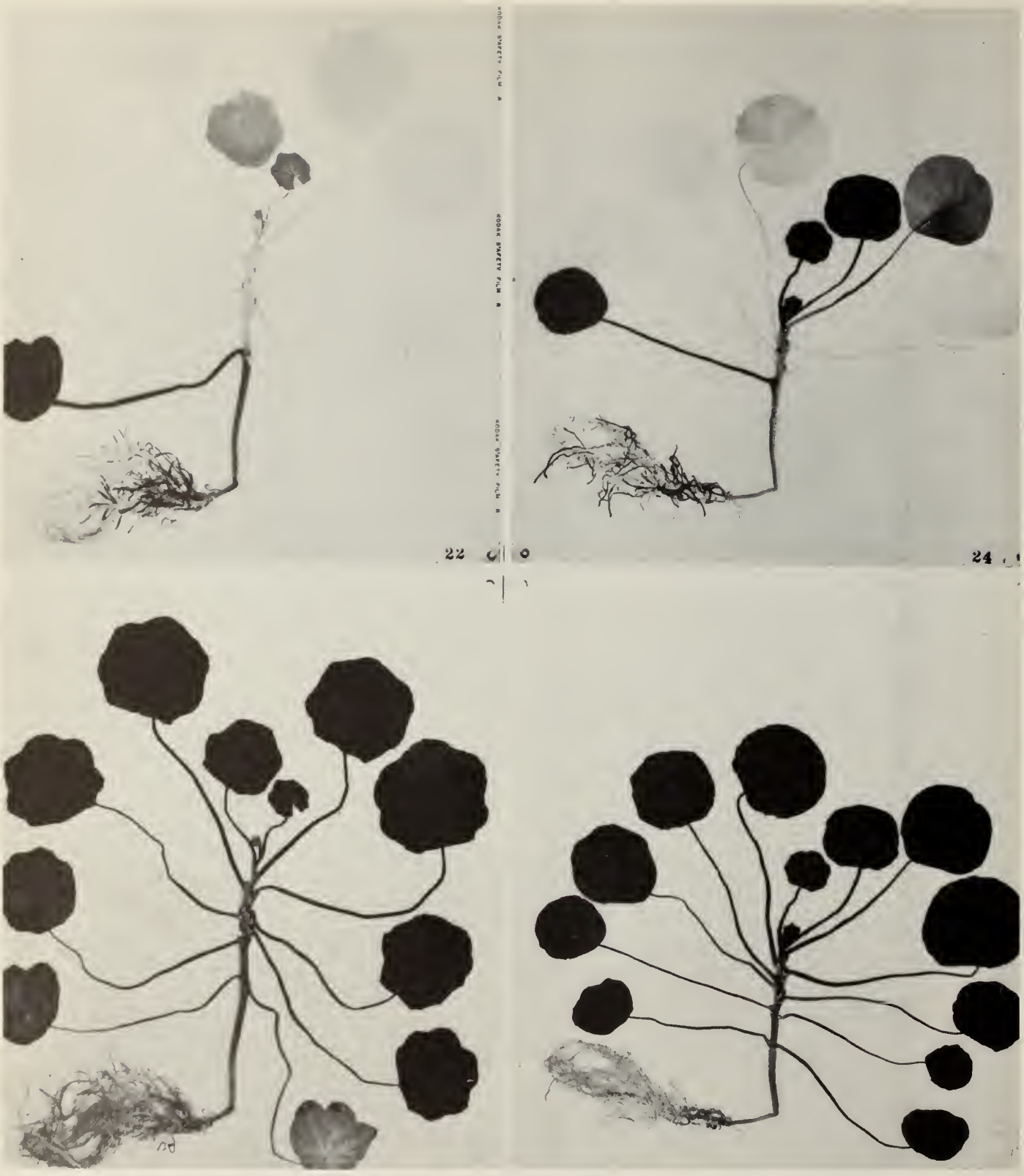


Fig. V, 6. Distribution of amitrole* applied to one of the lower pair of leaves of Tropaeolum majus (left) and to leaf 4 (right).

leaked from phloem to xylem in small amounts; a faint image of all leaves could be detected. Density of labeling of leaves along the axis of nasturtium can be used as a measure of compensation; leaves that were still enlarging were labeled, density being in proportion to their growth rate. In the plant treated on leaf 2, three young leaves at the shoot axis were densely labeled, and four leaves between the treated leaf and the first strongly labeled leaf were faintly

Fig. V, 7. Distribution of amitrole* applied to leaf 6 (left) and to leaf 9 (right) of T. majus.

labeled, apparently due to leakage.

From leaf 3, movement was about equally divided, the root system and three young leaves having dense labeling with the first pair and other mature leaves being virtually bypassed. Leaves 4, 5 and 6 (fig. V, 7, left) showed similar distribution of the tracer, with the balance of distribution shifting upward but the roots still showing strong labeling of the tips and late-formed adventitious members. Two plants were treated, each on leaf 9; the one on which this leaf was approaching full size showed two-way movement, although the roots, lower stem, and lower leaves were lightly labeled. The other plant (fig. V, 7, right) was treated on a small leaf; although it enlarged considerably during the 4-day treatment period all movement was acropetal, with two younger leaves being labeled. Most of the tracer was localized within the treated leaf.

EFFECT OF SHADING ON 2,4-D* TRANSLOCATION FROM LEAVES AND ROOTS

Work with cold 2,4-D soon after the discovery of its herbicidal effects proved that shading leaves to deplete them of stored foods will inhibit movement from them. In order to check this, an experiment using labeled 2,4-D on bean plants was carried out. Dosage was 1/10 µmole of tracer at 1.24 mc per mmole specific activity in 10 µl droplets; the same tests and dosage were also run with root treatments in 100 ml of Hoagland's solution. All plants were in the dark through the 24-hour treatment period, and the radiographs show that translocation from leaves was appreciably inhibited by pretreatment in the dark. Figure V, 8, shows plants pretreated for 6 hours (left) and 18 hours (right) in the dark. Figure V, 9, shows 30- and 42-hour dark pretreatments. Similar pretreatments had a slight but detectable effect on root uptake, but no effect on xylem movement from roots to tops; this last observation applied also to cotton, soybean and barley plants.

If one leaf of bean is kept in the dark and the opposite leaf treated, more tracer will enter the shaded leaf. When two bean plants were disbudded to hold down apical growth the treatment induced continued growth of the primary leaves. These plants were leaf-treated with 1/10 µmole of amitrole* of specific activity 0.94, a 10 µl droplet each. One plant was in the open greenhouse with both leaves exposed, and the untreated leaf of one plant was in the dark for 3 days before treatment and during the 4-day treatment period; evidently it became depleted, as more tracer entered it than entered the unshaded leaf (fig.V,10). Anderson (1958) has shown this effect in using amitrole on *Cyperus rotundus*.

The present data are offered as evidence that a mass-flow mechanism is responsible for rapid longitudinal movement in the phloem. How molecules moving by diffusion, or by any other mechanism involving random distribution, could consistently pass along stems while bypassing mature leaves and entering young expanding leaves and growing shoot tips or root systems, is difficult to comprehend. The distribution patterns noted above so obviously follow those of assimilated foods in the plant that the only plausible explanation seems to be a concomitant movement of tracer and food and water molecules in the assimilate stream, via the phloem, from the source of foods to the various sinks.

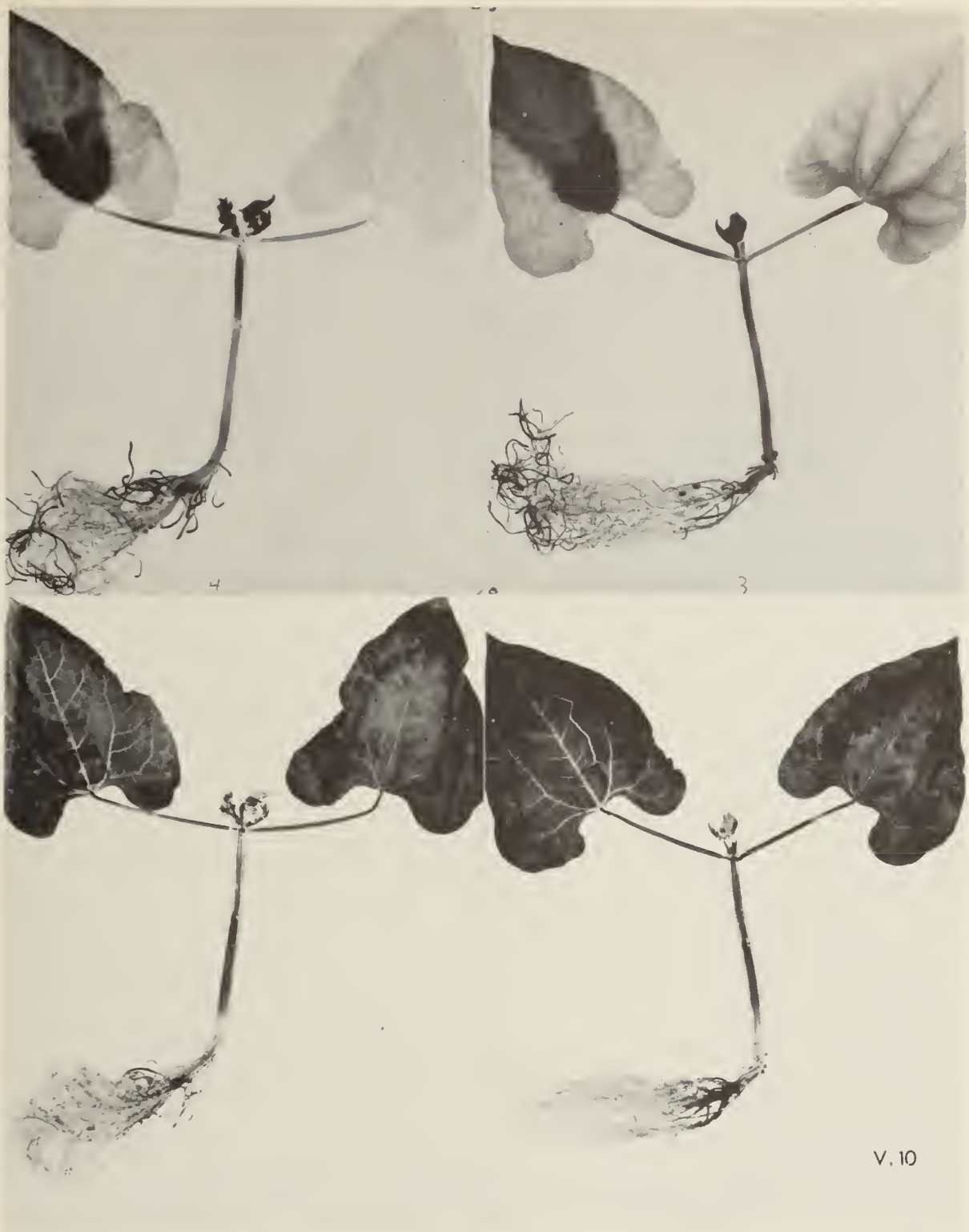


Fig. V, 8, opposite, right: effect of dark pretreatment on translocation of 2,4-D* in bean. From left: plant 1, root treatment; plant 2, leaf treatment 6 hours in dark; plant 3, 18 hours in dark; plant 4, 24 hours in dark. All plants treated in dim light and given 24-hours treatment time in dark.

Fig. V, 9, opposite, left: effect of dark pretreatment on translocation of 2,4-D in bean. Left pair of plants 30 hours in dark; right pair 42 hours in dark. All plants, 24 hours treatment in the dark.

Fig. V, 10, above: effect of dark treatment on translocation of amitrole* in bean. Plants debudded; left plant, opposite leaf in light; right plant, opposite leaf in dark.

Chapter VI. Comparative Movement of Tracers

With the synthesis of a number of C^{14}-labeled herbicides, tracer studies comparing the mobility of these compounds became possible. The first such experiment using 2,4-D*, amitrole*, and MH* has been described in a number of publications (Crafts and Yamaguchi, 1958; Crafts, 1961*a*, *b*). Figure VI, 1, shows the mounted plants and the autoradiographs of this experiment. The plants were growing in depleted soil and were about at a standstill, although healthy-looking. Two treatment times were used - 7 hours, and 4 days. The 7-hour treatments resulted in very little movement from treated leaves; the 4-day plants showed the interesting differences seen in figure VI, 1. Treatments in this experiment involved droplet applications of 10 µl each on three leaves of each plant; total dosage per plant was 0.5 µc.

The autographs show the inherent differences between these three compounds with respect to mobility in plants. The 2,4-D* remained accumulated mainly in the three treated leaves; little tracer was present in the stem beyond the points of attachment of these leaves. In these rather inactive plants 2,4-D* was relatively immobile, but in 4 days the amitrole* had moved throughout the plant and was present at low concentration in all stems and in the roots. It accumulated in three separate shoot tips and in one fruit at concentrations above that of the intervening stems; it bypassed many mature leaves.

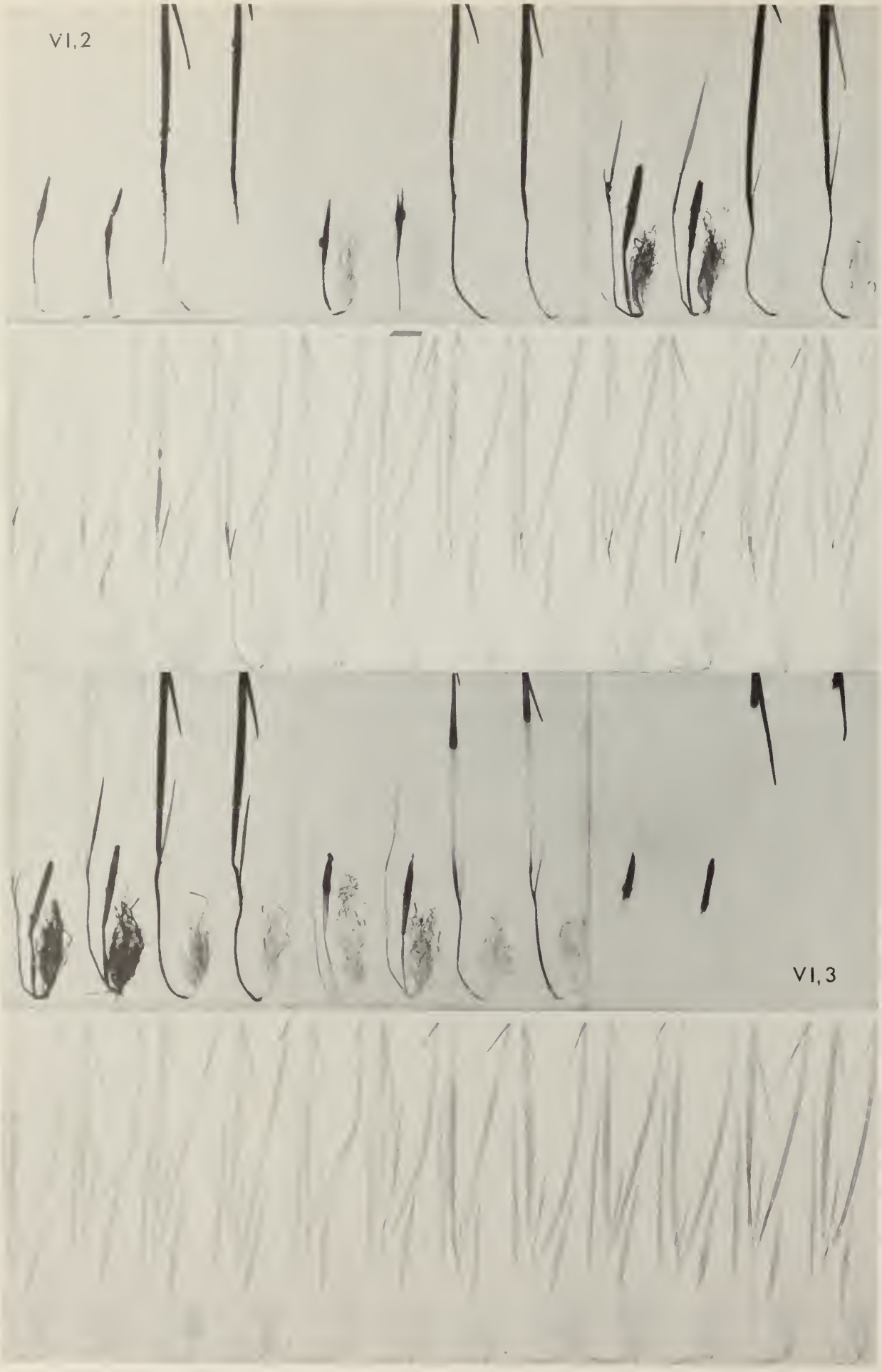
In the 4-day treatment period, the MH* had moved throughout the plant; it was present at medium concentrations in the stems from shoot tips to roots, decreasing from the treated leaves toward the base of the plant. Three shoot tips were intensely labeled, indicating normal phloem transport to active sinks. In contrast to amitrole*, MH* was present in all mature leaves in varying concentrations depending upon proximity to treated leaves. Intensity of labeling was lower in treated leaves than was the case in the 2,4-D*-treated plant

From these results it seems obvious that MH* moves most freely in a plant, amitrole* is intermediate, and 2,4-D* is least mobile. Since MH* moved so freely it seems that the limited movement of 2,4-D* reflects not a failure of the translocation mechanism but rather, a strong affinity for retention of these molecules by cells of the living leaves and stems. Accumulation apparently immobilized the chemical before it had opportunity to translocate any great distance in the slow-moving assimilate stream of these inactive plants.

Amitrole* moved throughout the plant body in this experiment but was limited to the phloem in the stem and roots. MH* apparently migrated from phloem to xylem and, carried by the transpiration stream, appeared in all leaves. The uniform labeling of mature untreated leaves indicates little tendency for MH* to be accumulated by living cells.

When a comparative experiment using 2,4-D*, amitrole*, MH*, urea* and monuron* was conducted on rape plants, the 2,4-D* moved more extensively, reaching the main stem in high concentration and the roots in amounts diminishing with distance.

Fig. VI, 1. Comparative movement of 2,4-D*, amitrole* and MH* in zebrina plants. Treatment involved 0.5 μc per plant in 75 μg of 2,4-D*, 150 μg amitrole* and MH*; treatment time, 4 days.


VI,2
VI,3

Amitrole* moved throughout roots and showed high accumulation in root tips; it bypassed one mature leaf, as did 2,4-D*. MH* moved effectively throughout the plant and was present in medium intensity in two mature leaves; again this is evidence for migration to the xylem. Urea* is rapidly hydrolyzed by urease in rape leaves; the $C^{14}O_2$ is synthesized to sugars, and chromatography shows the labeled compounds in roots to be sucrose. Mature leaves on this plant were bypassed; apparently sucrose is retained within the symplast. Monuron* apparently failed to enter the symplast; its distribution indicates apoplastic movement only, in treated leaves of tomato (Haun and Peterson, 1954), rape, barley (Crafts, 1959*b*) and several other plants.

The more extensive movement of 2,4-D* in the rape plant may be accounted for by two facts: the plants were young and actively growing, and the treatment time was 2.5 days - a shorter time than in the case of the zebrina.

An additional study was made using barley plants grown in nutrient cultures in a greenhouse. When they were at a stage in which there were four expanded leaves, 2,4-D*, IAA*, amitrole*, MH*, urea*, and monuron* were applied to leaf 1 on two plants and to leaf 4 on two plants. Dosage was 0.5 μc per plant of solutions standardized at 0.5 mc per mmole; treatment time was 27 hours.

Figure VI, 2, 3, show the mounted plants and autographs of this experiment.

Fig. VI, 2. Barley plants given 27-hour treatments with 2,4-D* (left 4 plants plants); IAA* (center 4 plants); amitrole* (right 4 plants). Of the four plants receiving each chemical, the left-hand pair were treated on leaf 1, the right hand pair on leaf 4. Dosage, 0.05 μc, volume 10 μl, specific activity, 0.5 mc per mmole.

Fig. VI, 3. Barley plants given 27-hour treatments with maleic hydrazide* (left 4 plants); urea* (center 4 plants); monuron* (right 4 plants). Of each group of four plants the left-hand pair were treated on leaf 1; the right-hand pair on leaf 4. Dosage 0.05 μc, volume 10 μl, specific activity 0.5 mc per mmole.

It is apparent that the first four compounds constitute a mobility series, increasing in mobility in the order given above. It is evident from the autographs that the older leaves provide nourishment for the roots, whereas the youngest expanded leaf provides for its own nourishment and exports to the younger leaves and inflorescence. Even in the case of MH* there is little evidence for leakage into the xylem, as the mature leaves show only in the case of the treatments on leaf 1. Amitrole* moved from leaf 1 to younger leaves, but bypassed all mature leaves.

Autographs of the urea* treatment indicate that urea is hydrolyzed during penetration, and that the CO_2* is rapidly incorporated into assimilates of which the principal constituent is sucrose which moves in the assimilate stream to regions of growth where food utilization is active. In the autographs of leaf 4 it is apparent that accumulation is high in the intercalary meristem and in the root tips, and low in the intermediary mature tissue.

Monuron* is not split by urease; it failed to move out of the treated leaves, and showed apoplastic movement only.

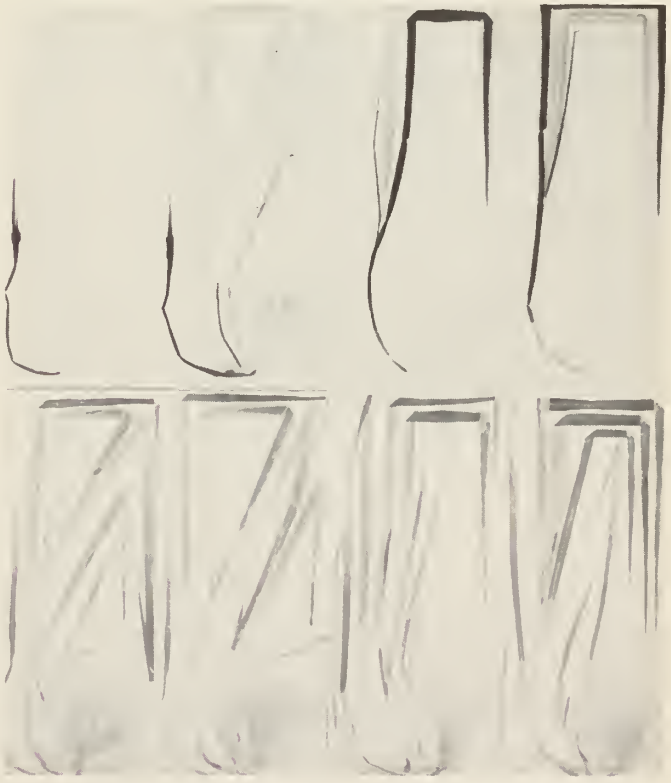


Fig. VI, 4. Barley plants treated with dalapon* on leaf 3 (left) and leaf 6 (right). Dosage was 0.1 μc per treatment.

Figure VI, 4, shows results of a similar experiment with dalapon* on somewhat older barley plants. Evidently this compound moves freely in the phloem; it also migrates to the xylem and shows apoplastic distribution, particularly from treatment on the older leaf. Noticeable also is the fact that dalapon* does not accumulate in the mature roots of the barley plants.

Since most of our work with labeled tracers had utilized whole intact plants where both absorption and translocation are involved, it seemed important to carry out tests in which these processes could be distinguished. To study absorption uncomplicated by rapid transport, a test using cubes of potato tuber tissue was employed.

Cubes were cut 1-1/2 inches square and placed on filter paper saturated with water in a metal container; a loose lid of cardboard was used as a cover. Lanolin rings about 7.0 mm in diameter were centered on the tops of the blocks and treatments were made using 10 µl per droplet in each ring, with 0.05 µc per application with solutions standardized at 0.5 mc per mmole. The chemicals used were 2,4-D*, amitrole*, MH*, urea*, monuron*, and IAA*; treatment times were 2, 4, 8, and 16 days. Each chemical was applied to two tuber blocks; at the end of the treatment time one block was split vertically and a 2 mm slice through the middle of the treatment was taken. The second block had three 2 mm horizontal slices removed, starting at the top. All slices were freeze-dried, mounted, pressed and autographed. In mounting, the three horizontal slices were inverted so that the autograph faces represented location of the chemicals at levels of 2, 4, and 6 mm in the tuber block. Figure VI, 5, shows the autographs and the tuber slices for the 2, 4, and 8-day tests (the vertical slices are on the left and the horizontal slices starting from the left are arranged in the order of 2 mm, 4 mm, and 6 mm).

In this storage parenchyma tissue in which phloem transport is not involved, the same mobility relation holds for 2,4-D*, IAA*, amitrole*, and MH*. 2,4-D* moved for the first two periods and then seemed to be immobilized. IAA* moved through the first three periods though not rapidly - in 16 days it had moved along the isolated phloem strands, presumably in phloem parenchyma because the sieve tubes were blocked (Crafts, 1933). Amitrole* continued to move slowly and by the 8th day the diameter of the occupied circle in the 2mm slice was about 2.5 cm. The MH* moved even farther, reaching the borders of the slice at the 2mm and 4mm level. Urea in this non-green tissue in the dark was apparently hydrolyzed and the CO_2* lost to the atmosphere. Monuron* showed typical apoplastic movement, traveling rapidly to the outer borders of the slices, where it accumulated as a result of evaporation of the aqueous medium. This is especially noticeable in the vertical slices, which show increasing concentration from the lower to the upper region as a result of the fact that the blocks stood on a water film from which movement caused by evaporation was upward and outward toward the exposed surface.

The experiments with potato tuber tissue show that differences in mobility between chemical compounds are not the result of differences in phloem transport *per se* but are differences in the ability of the molecules to migrate through relatively undifferentiated tissues such as the mesophyll of the leaf, the parenchyma of the root cortex, and the parenchyma of the vascular tissues. Apparently, parenchyma cells have different affinities for these tracer molecules; 2,4-D* seems to be absorbed with avidity and retained. This probably explains its failure to move from roots. IAA* moves a bit more freely; amitrole* moves relatively freely but is retained within the symplast; MH* is freely mobile. In whole plants it even leaks from phloem to xylem and ascends to the foliage in the transpiration stream; there is evidence that P^{32} may do this and thus circulate in plants (Biddulph, *et al.*, 1958).

If 2,4-D* can be retained by the chlorenchyma of the leaf it may be absorbed from phloem along the translocation route; this probably explains its limited distribution. When the assimilate stream is moving rapidly, as in the case of the plants used in experiments on leakage from roots, transport may be effective in moving 2,4-D into roots. When it is slow, the tracer is all absorbed and re-

tained within the treated leaves and stems and none reaches the root.

These conclusions have important implications in the use of 2,4-D for controlling perennial weeds; the recommendation of treatment at the early flower bud stage probably relates to the physiological condition when root carbohydrates are low (Weise and Rea, 1962), photosynthesis is high, and translocation rapid. Dosage of 2,4-D must pass through a maximum; Weise and Rea found this to be 1 pound per acre for field bindweed. The reason is that increasing dosage brings about increasing penetration, and translocation in the assimilate stream increases up to a point where contact injury impairs the source and transport stops. Optimum dosages for treatment of perennials with 2,4-D may vary widely because of differences in cuticle thickness, toughness of leaves, partition effects, saturation levels, and specific sensitivity to phytotoxicity, but optimum treatment levels have been found for many weed species.

MECHANISM OF ESTER TRANSPORT

In order to throw some light on the problem of how esters of the phenoxy herbicides move in plants, isopropyl esters of 2,4-D labeled on the carboxyl-carbon and on the alcohol chain with C^{14} were synthesized. When applied to cotton and barley plants (Crafts and Foy, 1959, Crafts, 1959*a*) it was shown that the ester is hydrolyzed during penetration of the leaf because the alcohol label was

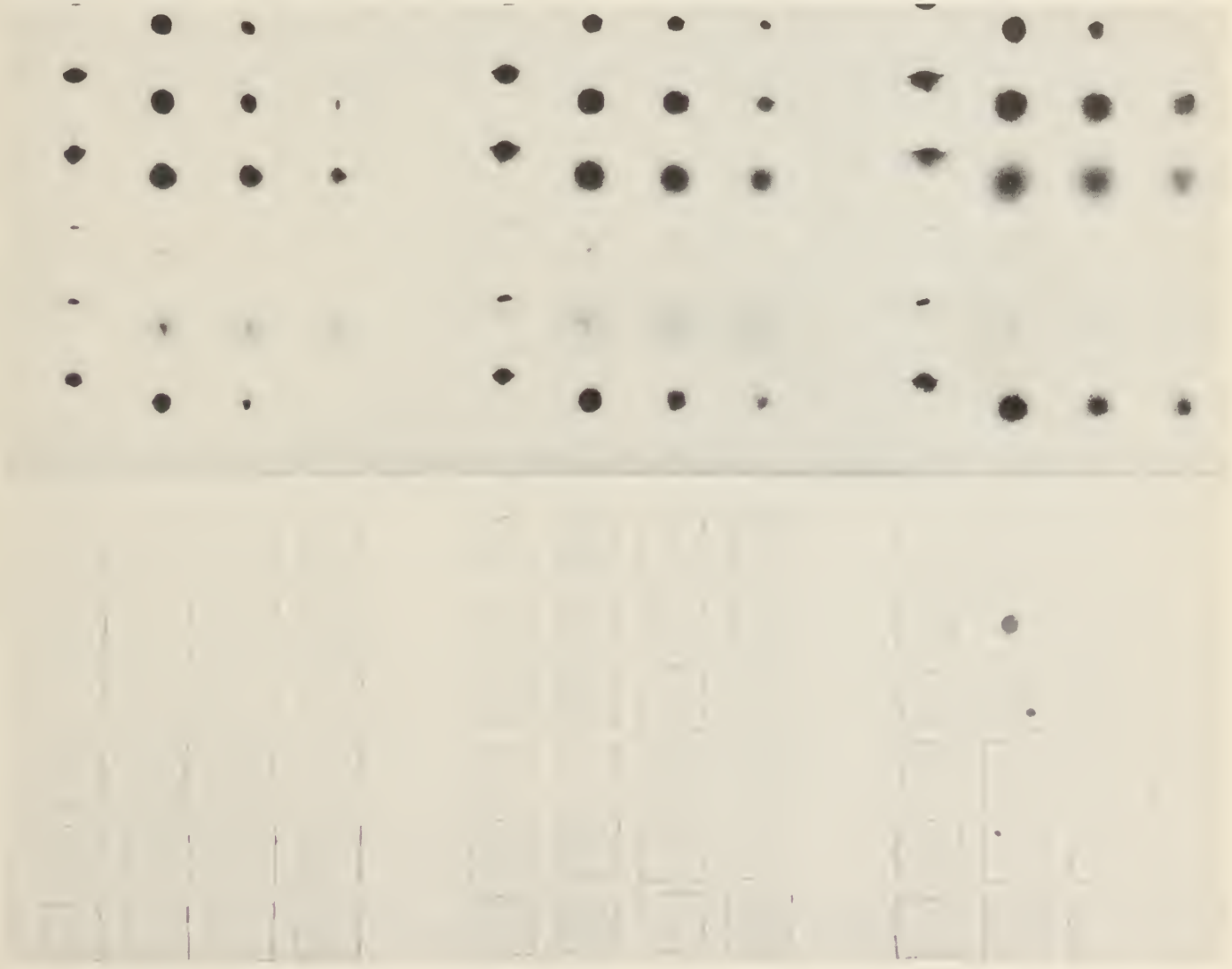


Fig. VI, 5. Tuber slices (lower) and autoradiographs (upper) of potato tuber blocks treated for 2, 4, and 8 days with, top to bottom, 2,4-D*, amitrole*, MH*, urea*, monuron*, and IAA*; no replication. Slices on the left of each treatment were taken vertically, those in the number 2, 3, and 4 positions are horizontal samples from another treated block taken 2, 4, and 6 mm from top surface.

subject only to apoplastic movement within the leaves, whereas the carboxy-labeled 2,4-D ion moved to the crown of the plant via the phloem just as did the ion from 2,4-D* acid treatment. Szabo (1963) has shown that hydrolysis of 2,4-D esters may take place in a solution in contact with the exterior leaf surface of some plants.

Labeling of molecules in different positions has proved extremely valuable in biochemical studies on metabolic pathways in plants; it was useful in studies on 2,4-D metabolism as employed by Weintraub, *et al.*, (1952*a*, *b*); it proved useful in this study on 2,4-D ester transport in plants, and has many more uses in studies on the uptake, distribution, and metabolic fate of herbicides in plants.

COMPARATIVE MOVEMENT OF LABELED TRACERS IN POTATO TUBER CYLINDERS

As new labeled compounds became available, it seemed desirable to study their movement into and through tissues of different types. Since movement of labeled compounds through parenchyma tissue may play an important role in their subsequent distribution in the plant, studies were made using cylinders of potato tuber tissue. Twelve C^{14}-labeled compounds plus P^{32} were applied to the upper ends of potato tuber cylinders within lanolin rings. Movement was determined by removing slices from the centers of the cylinders, freeze-drying, mounting and autographing. Table VI, 1, presents data on the penetration of the compounds into the cylinders.

Tissue from the tuber cylinder not used for autograph slices was freeze-dried, ground and extracted. From 96 to 97 per cent of the activity of 2,4-D* was extracted in alcohol and from one-fifth to one-sixth of the activity in the extract was not partitionable into chloroform - this was not 2,4-D*, and chromatographic studies indicated it to be a complex of 2,4-D* adsorbed to an alcohol-extractable substance or in weak conjugation; the amount of this substance was constant from 2 through 16 hours.

Studies on the amitrole* content of the dried potato tissue showed decreased extractability in alcohol with time; chromatography showed decreasing activity in the spot coincident with amitrole* with time. Only about 10 per cent of the alcohol-extracted activity was free amitrole*. A major fraction of the extracted activity remained near the origin and was associated with a dark-colored substance; this substance remained constant with time, being present at zero time. The other major fraction of the extractable activity was found in the extract from the residue of the treated samples and of the control (0 time), after partitioning with chloroform.

MH* was largely extracted with alcohol and the extract chromatographed as a spot coincident with stock MH-C^{14}. This compound seems to be almost completely immune from breakdown or conjugation.

Monuron* showed a drop in extractability, with time from 100 per cent at zero time to 91 per cent after 4 days. Monuron* was readily freed from movement interference by dark-colored materials; when the front was eluted and the eluate co-chromatographed with stock monuron* on reversed phase paper, the only spot of significance was monuron*.

Small amounts of simazine* moved into the potato tissue; simazine-C^{14} decreased with treatment time from nearly 100 per cent at zero time to about 25 per cent at 16 days. The remaining activity occurred in three other spots with lower Rf values; one of these was associated with dark-colored materials, and a third spot was coincident with a radioactive impurity present in the original simazine*.

Alcoholic extracts of IAA*-treated tissue showed no IAA-C^{14} after 4 days.

COMPARATIVE UPTAKE AND DISTRIBUTION OF TRACERS APPLIED TO ROOTS

Because tracers applied to leaves are subject to such wide differences in distribution in plants it seemed logical to study comparatively the uptake by roots. Following a preliminary trial with barley seedlings growing in small culture tubes, an extensive test using 2,4-D*, IAA*, amitrole*, MH*, urea*, dalapon*, monuron*, simazine*, and P^{32} was set up. Each plant used was growing in 4 ml of culture solution and dosage was standardized at

0.037 μc per plant. The following were the treatment times: 1/2-hour, 1 hour, 2 hours, 4 hours, 8 hours, 16 hours, 1 day, 2 days, 4 days, and 8 days. The roots were rinsed and the plants freeze-dried, mounted and autographed (Crafts and Yamaguchi, 1960*a*). Figures VI, 6, 7, 8, show the results of this experiment.

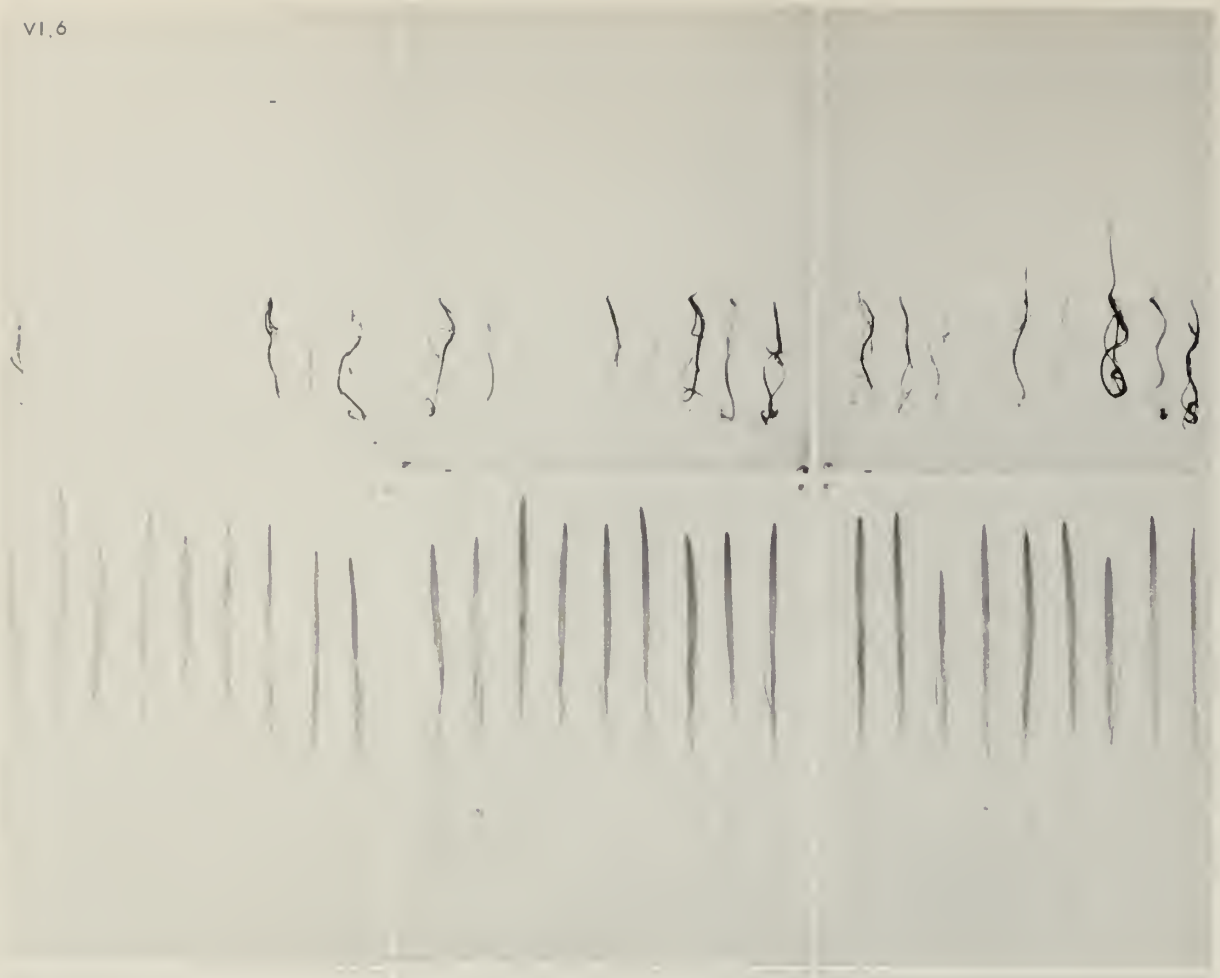
The 2,4-D*, monuron*, simazine*, and IAA* were absorbed sufficiently in 30 minutes to give strong images of the roots. In 2 hours amitrole* and P^{32} had also entered the roots sufficiently to give good autographs. Monuron* and simazine* started moving upward in 2 hours and by 8 hours the tops were all labeled. In 16 hours, amitrole*, dalapon*, and P^{32} had concentrated sufficiently in tops to autograph well; monuron* and simazine* continued to accumulate. P^{32} built up to high concentrations in tops and roots so that in 2, 4, and 8 days the images of the roots became broad and blurred (this results from the high-intensity radiation of P^{32}). After 4 days very little 2,4-D*

TABLE VI, 1
Penetration of Labeled Compounds into Median Slices From Potato-tissue Cylinders

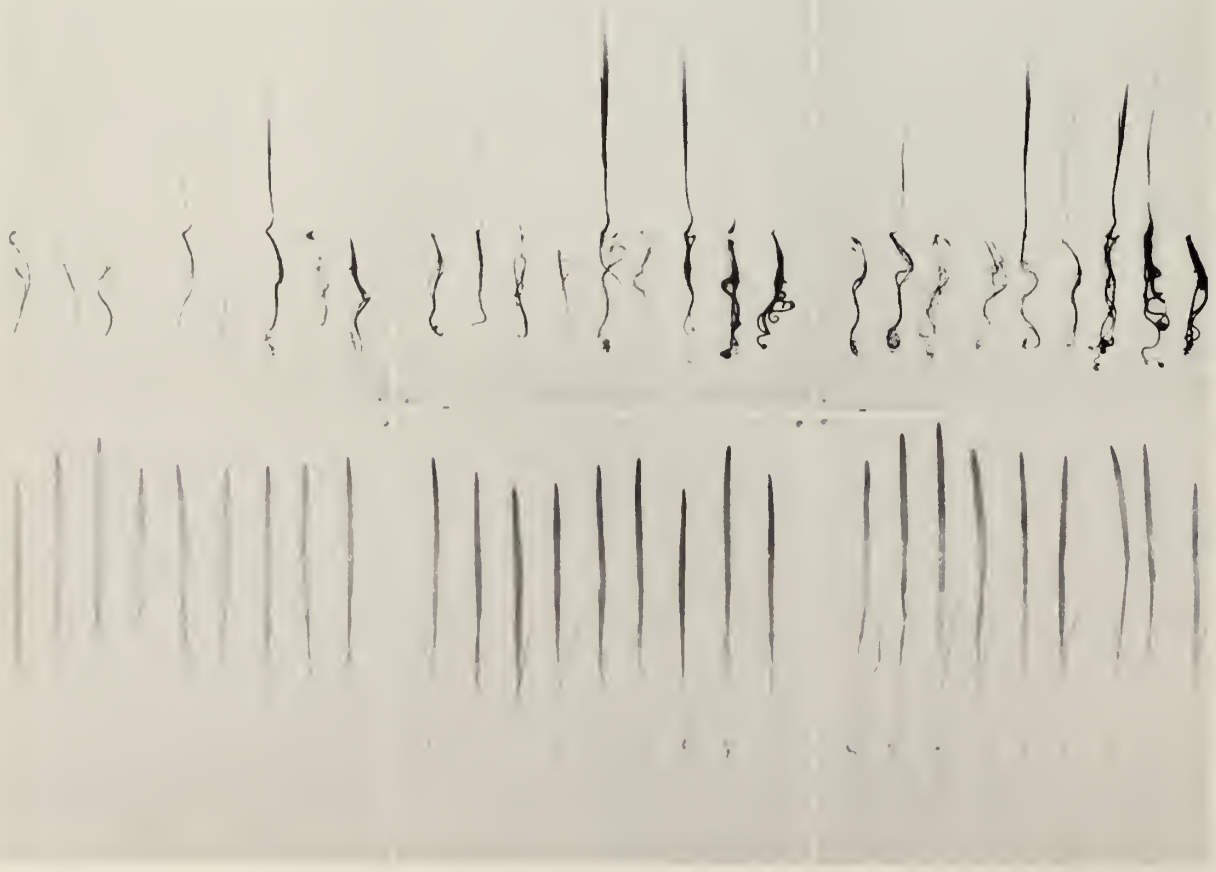
Compound	Penetration (millimeters)				
	Days				Average penetration
	2	4	8	16	
Urea	2	1	1	1	1.25
Alanap	2	3	5	5	3.75
Duraset	5	5	5	6	5.25
Simazine†	5	6	5	5	5.25
2,4-D	7	7	6	7	6.75
2,4,5-T	7	6	8	8	7.25
Sodium benzoate	8	6	8	7	7.25
IAA†	5	6	10	11	8.0
Sodium phenylacetate	8	10	8	10	9.0
Amitrole	9	11	15	20	13.5
IAA‡	10	11	15	20	14.0
$P^{32}O_4$	12	10	20	18	15.0
Simazine§	12	15	20	23	17.5
MH	10	15	20	35	20.0
Monuron§	23	34	45	55	39.0

† Penetration in symplast
‡ Penetration in phloem strands
§ Penetration in apoplast

VI.6



VI. 7



and urea* had entered the tops, and after 8 days these tracers and IAA* were still in low concentration in the foliage. Urea* was still low in intensity in the roots; IAA* and 2,4-D* were concentrated in roots.

From these studies it seems obvious that even though different tracer molecules enter leaves and roots at different rates and attain different distributions, the patterns of movement reflect the two basic mechanisms - that is, symplastic movement via parenchyma cells and phloem, and apoplastic movement via cell walls and xylem. Although different molecules such as 2,4-D, IAA, monuron and simazine enter roots rapidly, it does not follow that they migrate into and move in the xylem with ease. 2,4-D and IAA, which are avidly accumulated by living parenchyma, were the slowest to enter the xylem of roots and move to tops; monuron and simazine, which follow an apoplastic pattern, moved most rapidly to the tops. Just how these latter molecules are able to traverse the root cortex, endodermis and pericycle so rapidly is unknown; they must enter and cross living cells in order to do this.

Fig. VI, 6. Comparative uptake and translocation of nine tracers by barley plants. The tracers in each set as follows, left to right: 2,4-D*, amitrole*, MH*, urea*, monuron*, dalapon*, simazine*, P^{32}, and IAA*. All tracers except P^{32}, C^{14}-labeled; each plant with roots in 4 ml of Hoagland's solution with 0.037 μc of tracer. Time series, left to right: 1/2 hour, 1 hour, 2 hours.

Fig. VI, 7. Comparative uptake and translocation of nine tracers by barley plants. Chemicals and conditions same as in fig. VI, 6. Time series, left to right; 4 hours, 8 hours, and 16 hours.

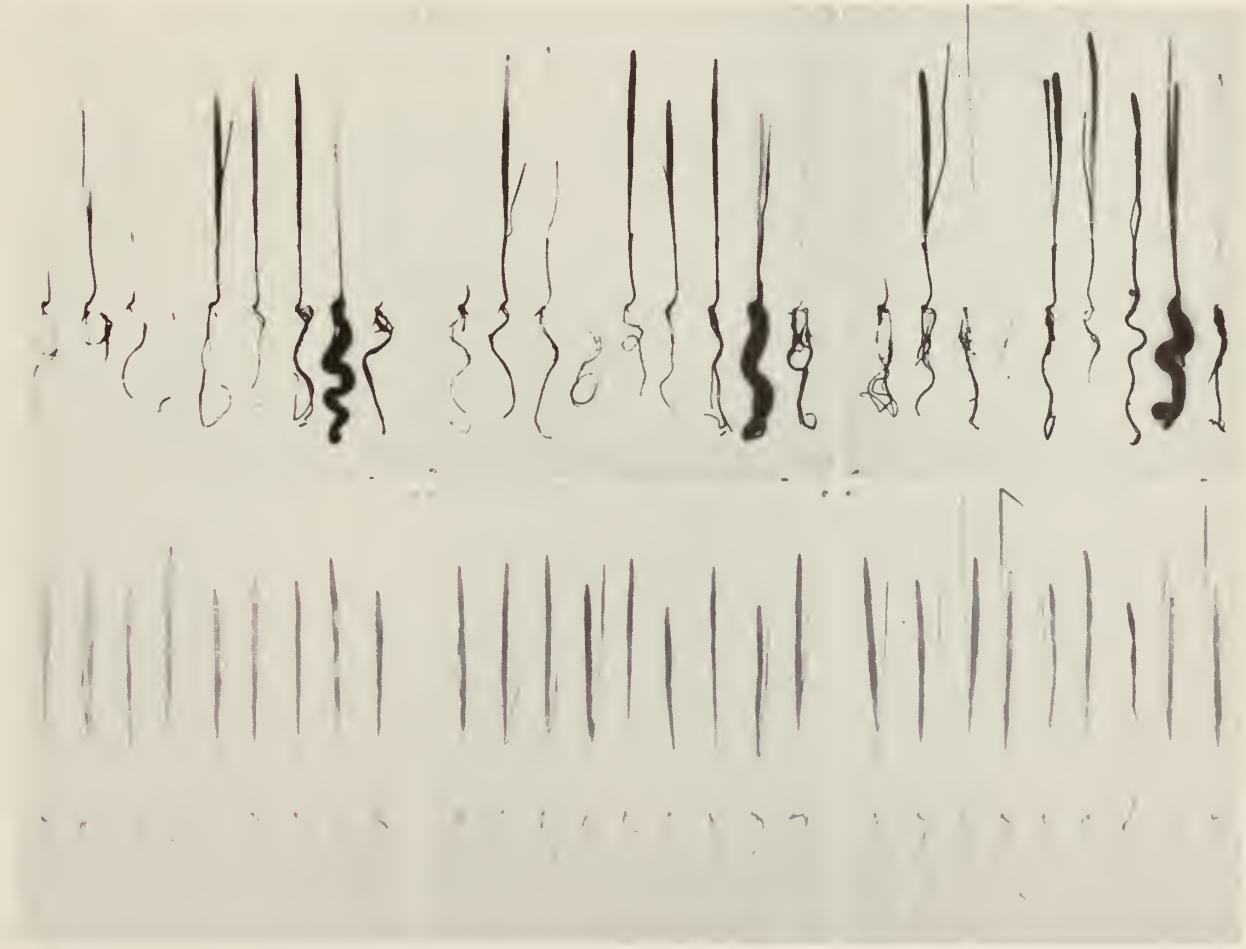


Fig. VI, 8. Comparative uptake and translocation of nine tracers by barley plants. Chemicals and conditions same as in fig. VI, 6. Time series, left to right; 2 days, 4 days, and 8 days.

Chapter VII. Root Absorption and Xylem Transport

MOVEMENT OF 2,4-D* ABSORBED BY ROOTS

Having determined to our own satisfaction the nature of the mechanism responsible for the translocation of 2,4-D* applied to leaves, it seemed important to study uptake and distribution by root absorption. Accordingly, plants of barley, bean, cotton and zebrina were grown in nutrient cultures; when they had developed a number of leaves per plant they were treated by adding 2,4-D* to the culture solution at a rate of 1.25 μc per 250 ml, each plant having its roots limited to 250 ml. The 2,4-D* had a specific activity of 5.0 mc per mmole. Treatment time was 10 days; at the end of this time the roots were rinsed in tap water and the plants freeze-dried and autographed. Figure VII, 1, shows barley plants and

Fig. VII, 1. Autoradiographs (left) and plants (right) of barley grown 10 days in 250 ml of solution containing 1.25 μc of C^{14}-labeled 2,4-D.

TABLE VII, 1
Radioactivity Recovered from Culture Solutions
In which 2,4-D*-treated Cotton Plants Were Grown

Replication	Radioactive counts per minute at various time intervals†			
	0-2 days	2-4 days	4-8 days	8-16 days
1 . . .	2,235	13,165	2,378	1,254
2 . . .	1,948	12,944	3,990	1,358
3 . . .	1,875	10,760	7,700	1,120
4 . . .	2,602	10,892	2,103	921
5 . . .	1,928	8,660	2,728	845
6 . . .	2,332	14,524	3,428	1,304
7 . . .	2,744	16,635	4,280	440
Average	2,238	12,511	2,801	1,035

† Activity is shown as counts per minute per plant within given time intervals. Plants were transferred to new culture solution at the end of each interval. Dosage 0.5 μc of 2,4-D* was applied to upper surface of one cotyledon.

autographs from the experiment.

In the 10-day period, 2,4-D* had accumulated on or in the roots but not much had translocated to the tops. Bean and zebrina plants apparently translocated none at all, barley translocated enough to show traces in the leaves, and cotton plants showed clear but faint images. This indicates that 2,4-D is not readily moved from roots to tops of plants, and may provide a reason why 2,4-D, when used in the soil, must be applied pre-emergence so that the compound comes in contact with the roots as soon as they emerge from the seeds; the results may also help explain why a high dosage of 2,4-D is required when it is used to kill grown plants.

LOSS OF 2,4-D* TO THE CULTURE MEDIUM

In 1951, Clor observed that when one of two cotton seedlings growing in a common culture jar was treated on a lower leaf with 2,4-D the other plant showed 2,4-D symptoms within about 2 weeks (Crafts, 1956*a*, fig. 1). To further check this phenomenon, barley, bean, cotton and zebrina plants were grown in water culture, with cotton seedlings included in the culture jars of barley, bean, and zebrina. Barley, bean, cotton, and zebrina plants in the cultures were treated with 2,4-D* at 0.5, 1.0 and 2.0 μc per plant, leaving the cotton seedlings untreated to serve as indicators of 2,4-D* leakage. All plants were freeze-dried and autographed after 24 hours. A similar set was left for 15 days in the case of barley, 20 days for bean, cotton, and zebrina. In all cases faint autographs were obtained for the 24-hour treatment times, and somewhat darker autographs for longer treatment times. The untreated cotton test plants in the latter experiment showed 2,4-D symptoms, and significant counts of the 2,4-D* concentration in some culture solutions were recorded. Clor (1959) has confirmed these results, as shown in tables VII, 1, 2.

It seems obvious from these experiments that 2,4-D* is absorbed by leaves of plants, that it moves to the roots and, under some conditions, may leak from the

roots into the culture medium. Later experiments have shown that maleic hydrazide will do this but that amitrole and sucrose will not; this indicates that roots respond differently to different compounds. Experiments cited in chapter VI prove that roots also respond differently to different compounds applied to them via the culture medium.

TRANSLOCATION OF LABELED HERBICIDES IN XYLEM AS INDICATED BY ACTIVITY OF GUTTATION FLUID

In order to further study the comparative responses of plants to a number of herbicides, an experiment was set up to find out if these compounds are altered during their absorption by roots and translocation to tops via the xylem. Barley seedlings were grown in culture solution in small tubes and, after the solutions containing the chemicals were introduced, guttation fluid was collected and droplets from ten plants were pooled to provide enough for chromatography. The plants were covered with bell jars in order to obtain sufficient fluid; at the end of the collection period the plants were freeze-dried, mounted and autoradiographed. Each plant was in a tube containing 4 ml of culture solution, and dosage was 0.05 μc per plant. The collection period was from 4 to 6 days.

The effects of the herbicides on plants were recorded at time of harvest. Plants treated with amitrole* and MH* were still guttating, those treated with 2,4-D*, monuron* and simazine* had stopped. In general, 2,4-D* stunted the plants, amitrole* caused chlorosis on the lower halves of the first leaf, and on the whole second leaf; MH* had no visible effect at the time and concentration used; monuron* reduced the size and growth-rate of all plants (the leaves were light green and wilted at the tips); simazine* had effects similar to those of monuron*.

2,4-D* was absorbed by roots but moved slowly to the tops. MH* accumulated slowly in roots but moved to tops in somewhat greater amount than did 2,4-D*. Amitrole* moved to tops in even greater amounts, the images attaining a dense black tone. All plants treated with monuron*,and simazine* were heavily labeled throughout, with a noticeable tendency for accumulation at the leaf tips in old leaves in contrast to young. This distribution pattern, a reverse of that of 2,4-D*, MH*, and amitrole*, is an indication of apoplastic movement.

Guttate was chromatographed from all collections. In the case of 2,4-D* treatment there was no spot at the normal Rf of 2,4-D* as shown on the control; there was a small, faint image at the solvent front and this corresponded with a similar spot on the control, indicating a small impurity in the 2,4-D* - this impurity came through in the exudate, whereas 2,4-D* did not. Amitrole* produced spots in all replicates and these corresponded to the amitrole* spot on the control; evidently it came through the plant unchanged. In the case of MH* some replicates produced no spots; three separate replicates gave faint images of the Rf value of the original compound. All replicates from the monuron* experiment gave images of moderate density; one replicate gave the strongest spot of all the samples. In the case of simazine*, chromatography of the original compound gave three distinct spots on the control; three replicates from the treated plant samples produced the same three spots.

Here, there did not seem to be any relation between the volume of guttate and the image density on the chromatograph.

TABLE VII, 2
Radioactivity Recovered from Culture Solutions In which 2,4-D*-treated Bean Plants Were Grown

Replication	Radioactive counts per minute at various time intervals†		
	0-2 days	2-6 days	6-14 days
1 . . .	220	1,268	286
2 . . .	184	928	116
3 . . .	195	1,451	366
4 . . .	312	1,016	325
5 . . .	378	1,384	223

† Activity as counts per minute per plant within given time intervals. Plants were transferred to new culture solutions at the end of each interval. Dosage 0.5 μc of 2,4-D* applied to the upper surface of one primary leaf.

2,4-D* failed to come through the plants, but the other four compounds traversed the roots and tops and appeared in the guttation fluid. There was no evidence for metabolic change of any compound despite the fact that they must have moved through the living cells of the endodermis. These observations confirm evidence from many sources that herbicides in general are stable compounds and that they probably cause their characteristic effects without being altered in the process.

THE MECHANISM OF ACCUMULATION OF 2,4-D BY ROOTS

The evidence presented above, and the work of Blackman (1955, 1957), indicates that 2,4-D accumulation by roots may be reversible. To study this, excised root systems of nutrient-grown barley plants were exposed to varying concentrations of labeled 2,4-D in aerated culture solution. After a 6-hour absorption period, concentration of 2,4-D* in the roots was from two to four times that of the external solution. A time series showed that under the temperature conditions of the experiment (approximately 70 degrees Fahrenheit) absorption was rapid and reached its maximum in 30 minutes. From washing and exchange experiments conducted at this time it seems that 2,4-D is rather loosely bound and can be readily removed by leaching. However, not all of the absorbed 2,4-D* came off so easily - possibly, some is adsorbed to the outer root surface and some is held metabolically by parenchyma cells of the root cortex.

Recent studies in which DNP (dinitrophenol) treatment was used in connection with the uptake of 2,4-D* by roots throw more light on the subject. Where barley roots had absorbed 2,4-D* from a solution of 0.1 µmole per 100 ml for 4 days, DNP applied simultaneously at 1×10^{-4} M caused a slight release to the tops; at 1×10^{-3} a large release to the tops occurred. With bean plants in a similar 2,4-D* solution, 1×10^{-5} M DNP released some 2,4-D* to the stem, and 1×10^{-4} released considerably more; a similar response was found in soybean. Figure VII, 2, shows plants and autographs from these experiments.

Since DNP is known to uncouple oxidative phosphorylation, thus reducing energy supply to the cells, it seems that 2,4-D accumulation in roots is an active process; lowering the energy supply and membrane integrity apparently allows 2,4-D to move across the cortex and endodermis and enter the xylem (apoplast) of the stele along with the transpiration stream. Current studies with high dosages indicate that 2,4-D applied to roots in injurious amounts will bring about the same free movement into tops. Figure VII, 3, shows an experiment with a 2-hour absorption time, the roots of the plant on left having been in a solution having 0.1 µM concentration, and those of the plant on the right in a solution having a 10.0 µM concentration per 20 ml. Figure VII, 4 shows a similar experiment with a 24-hour exposure time. Monuron* and simazine*, being less subject to active accumulation by living cells, can apparently pass through the root with little restraint. Hence, these compounds readily move through roots and stems and accumulate in leaves where, if in sufficient concentration, they enter green cells and block oxygen evolution in photosynthesis (Crafts, 1961*a*). The plants in this experiment were alive at the end of the 24-hour absorption period; evidently 2,4-D* was being metabolized and lost from the leaves.

DINITROPHENOL EFFECTS ON HERBICIDE TRANSPORT

Many tests on the uptake of herbicides by roots and translocation to tops have proved that there is a wide variation in distribution. Although 2,4-D* is highly accumulated by roots with little or no movement into tops, monuron*, simazine*, calcium* and phosphorus* are rapidly translocated following a short accumulation period. Experiments with DNP proved that this respiration inhibitor, when used at a concentration of around 10^{-4} M, would affect roots in such a way that 2,4-D* was released for transport to the foliar region of the plant.

Repetition of the above tests with sufficient replication to assure significant results proved that DNP at 10^{-4} M will allow for transport of 2,4-D* to the tops of soybean plants, and that it has a slight but detectable effect in barley and little effect in bean. At

10^{-3} M concentration it has a strong releasing effect on 2,4-D* in all three species, but it also produces injury symptoms; at 10^{-5} M DNP, soybean is the only species tested that gives a detectable difference - and it is at the lower limit of significance.

DNP had no effect upon the movement of amitrole* from the roots of soybean at 10^{-4} M; MH* movement was slightly inhibited in soybean and barley, as was P^{32} and Ca^{45} movement in barley; monuron* transport was unaffected in soybean and barley. Figure VII, 2, shows a 6-hour treatment of soybean plants cultured in Hoagland's solution with 2,4-D*, at 1/2 µmole per 100 ml, with DNP at 10^{-4} M compared with no DNP. In a series of barley cultures, sodium benzoate* at

Fig. VII, 2. Effect of dinitrophenol on the movement of 2,4-D* from roots of soybeans. Left, soybean cultured 6 hours in 2,4-D* solution at 0.1 µmole per 100 ml. Right, soybean cultured 6 hours in 2,4-D* at 0.1 µmole per 100 ml plus DNP at 10^{-4} M.

Fig. VII, 3. Effect of high concentration of 2,4-D* on movement through the root system. Left, bean plant cultured in a solution of 2,4-D* at 0.1 μmole; right, the same plant in a solution of 2,4-D* at 10 μM concentration per 20 ml. Treatment time, 2 hours.

Fig. VII, 4. Bean plant in a solution of 2,4-D*. Effect of high concentration of 2,4-D* on movement through the root system. Plants and treatments as shown in fig. VII, 3, but with a 1-day treatment. The lower concentration of tracer in leaves may result from decarboxylation of the 2,4-D*, from translocation back to the roots, or from both.

1/10 μmole in 100 ml for 4 days showed no upward movement; 2,4-D* at the same dosage shows detectable movement; monuron* shows strong upward transport but no response to DNP; P^{32} and Ca^{45} show strong upward movement inhibited by DNP; MH* shows weak upward movement slightly enhanced by DNP, but accumulation in the roots is noticeably reduced. Figures VII, 5, 6, 7, show these results. Sodium azide affects 2,4-D movement, as does DNP.

Repeated trials with bean, barley, soybean and buckwheat failed to give any positive results when DNP was applied with 2,4-D*, 2,4,5-T* or monuron* to leaves. Apparently, movement from cortex to stele in roots is governed by different factors than is migration from cuticle to symplast in leaves; the comparative behavior of the substituted urea and triazine herbicides when applied to leaves and roots substantiates this belief. Trials with sodium fluoride proved that this inhibitor has no effect on 2,4-D* uptake by roots or movement from roots within the concentration range of 10^{-2} M to 10^{-4} M in soybean. Other metabolic inhibitors are being tested.

FIGURES VII, 5, 6, AND 7 FOLLOW. ▶ ▶ ▶

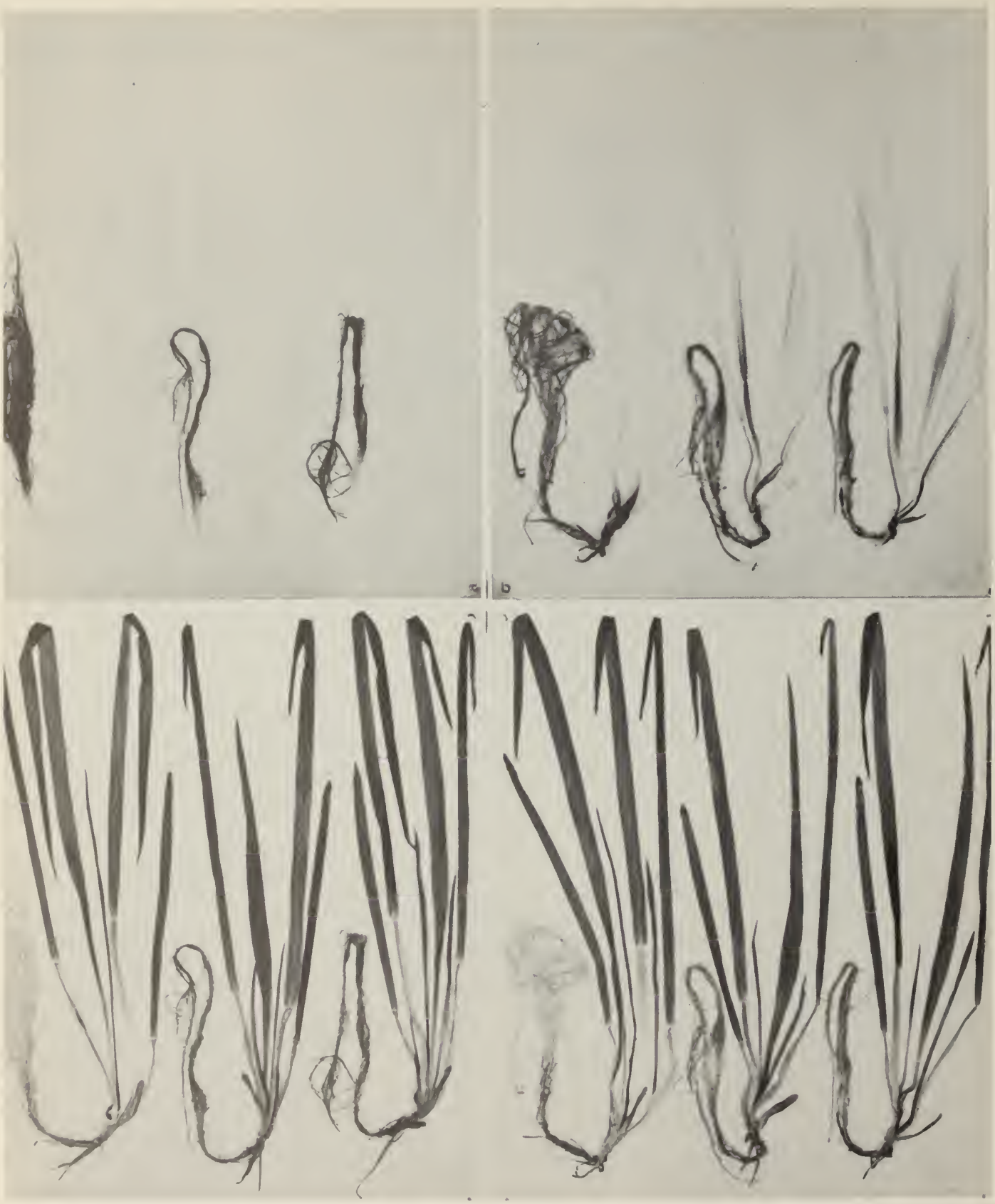


Fig. VII, 5. Effect of DNP on the uptake and transport of sodium benzoate* (left), and 2,4-D* (right). Plant on left in each set of three plants is the control, the other two were treated for 1 day with DNP at 10^{-4} M. DNP had no effect on sodium benzoate*, but released 2,4-D* for movement to tops.

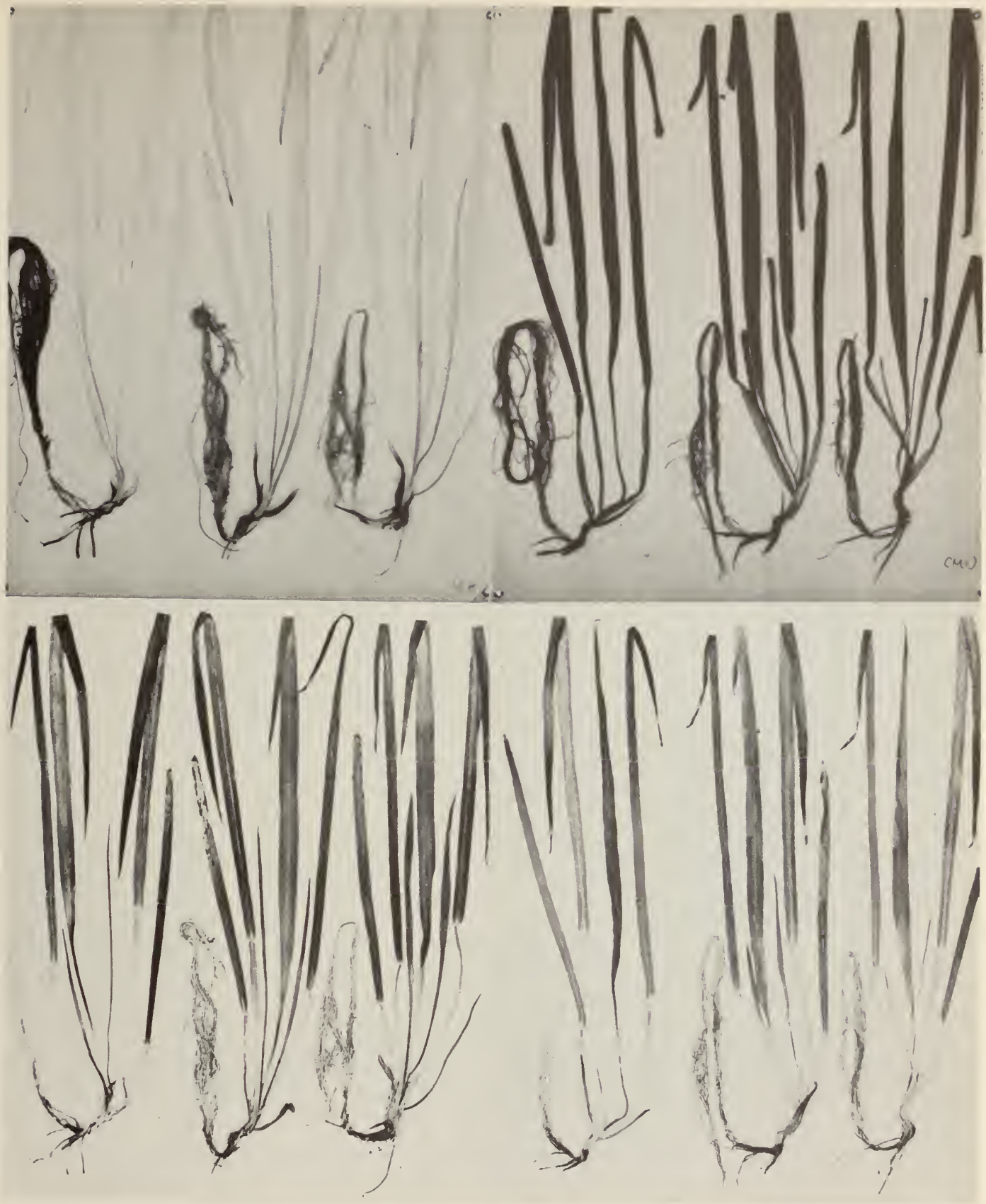


Fig. VII, 6. Effect of dinitrophenol on uptake and transport of maleic hydrazide* (left), and monuron* (right). Plants and treatments as in fig. VII, 5. MH*, like 2,4-D*, was released to the tops; there was no observable effect on monuron* movement.

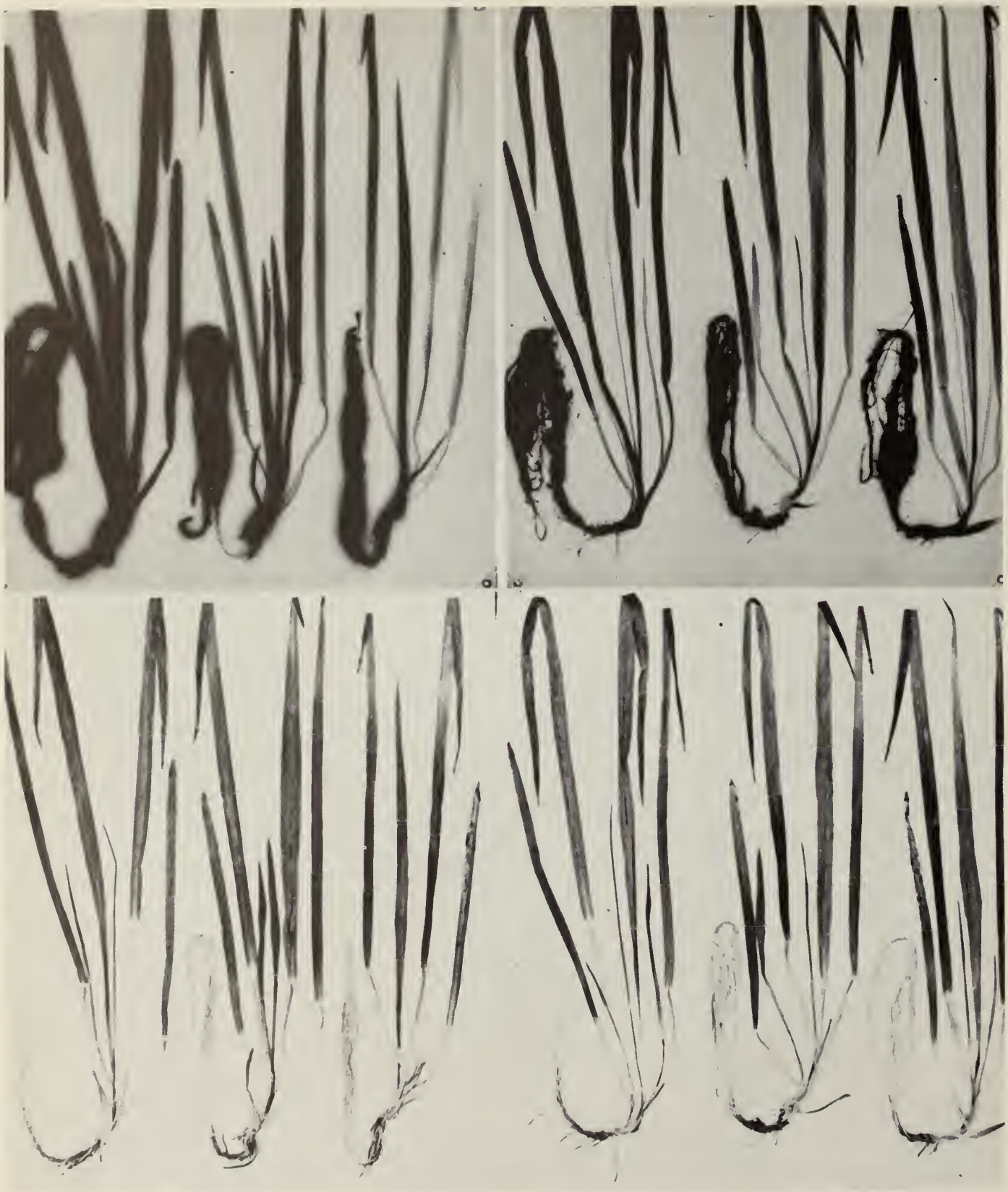


Fig. VII, 7. Effect of dinitrophenol on uptake and transport of P^{32} (left), and Ca^{45}(right). Plants and treatments as in fig. VII, 5. DNP apparently inhibits the uptake and movement of these inorganic elements.

Chapter VIII. Comparative Movement of Tracers via Shoot and Root

MOBILITY OF FOUR TRACERS IN BARLEY APPLIED TO LEAVES AND ROOTS

To compare leaf and root application and to detect root leakage in barley, an experiment using 2,4-D*, IAA*, amitrole*, and simazine* was carried out. To study initial absorption, translocation and redistribution, the plants were given 1-day, 4-day, and 16-day treatment times. To test for root leakage, three plants were grown in a single culture jar; two received applications on their leaves, and one was left to indicate leakage.

In the foliage treatments, 2,4-D* moved very little below the root crowns even in the 16-day period, while IAA* was appreciably more mobile, giving light images of roots from the leaf treatments at 4 days and similar images in 16 days. MH* was quite mobile, giving heavy images after 1 day and continuing to move even up to 16 days; there was ample evidence that MH* retranslocated within the plants during the 16-day treatment period. Simazine* failed to move basipetally from the treated spots on the leaves at any treatment time; movement in foliage was wholly apoplastic.

The untreated test plants were free of tracer except in the MH* leaf treatments; the roots of the test plant produced a light image in 4 days; after 16 days the whole plant was labeled.

In root treatments, 2,4-D* failed to leave the roots, while IAA* moved upward in 4 days producing an image of medium intensity; in 16 days the whole plants were heavily labeled. MH* failed to leave the roots in 1 day; light images of tops were produced in 4 days; in 16 days, larger tops were thoroughly labeled, but only at low intensity. Simazine* moved to the tops, and produced intense images in all treatment times. Figure VIII, 1, shows the 1- and 16-day plants and autographs of the 2,4-D*, IAA*, and simazine* experiments.

Bean and barley plants were next treated by leaf application with 2,4-D*, amitrole*, MH*, dalapon*, P^{32}, Zn^{65}, and Ca^{45} using treatment times of 1, 4, and 16 days. In this experiment the relative mobility of 2,4-D*, amitrole*, and MH* was consistent with previous tests. Dalapon* proved very mobile; like MH*, it migrated from phloem to xylem and thus the untreated opposite leaves of bean were lightly labeled. From leaf treatment as shown by figure VIII, 4, right, dalapon entered the roots but failed to accumulate. Resembling MH*, it probably leaked into the ambient culture medium and migrated to the xylem - as evidenced by the labeling of untreated mature leaves. Because grass roots are unable to produce shoot buds, and hence are not vegetatively reproductive from root tissue, this response to dalapon may explain its selectivity against grasses; in grasses, killing of stems and buds is all that is necessary to destroy the plant.

P^{32} and Zn^{65} proved to be phloem-mobile in barley and bean; they translo-

Fig. VIII, 1. Autoradiographs (top) and mounted plants (bottom) showing the distribution of 2,4-D* (a, d), IAA* (b, e), and simazine* (c, f) applied to leaves (left) and roots (right) of barley plants. Top row (a, b, c) 1-day treatment times; bottom row (d, e, f) 16-day treatment times. Dosage 0.1 μc applied to the 4th leaf in each case; 1.0 μc in 200 ml of solution to roots of 3 plants in the root treatments. ——→

a.
b.
c.
d.
e.
f.

cated freely and redistributed to young leaves in the 16-day period. Ca^{45} was mobile only in the apoplast; like monuron* and simazine* this element produced a strong pattern of apoplastic movement in leaves but failed to move out via phloem even in 16 days. Figures VIII, 2, 3, show the plants and autographs from the 1-day part of this experiment; figure VIII, 4, illustrates the evidence for leakage

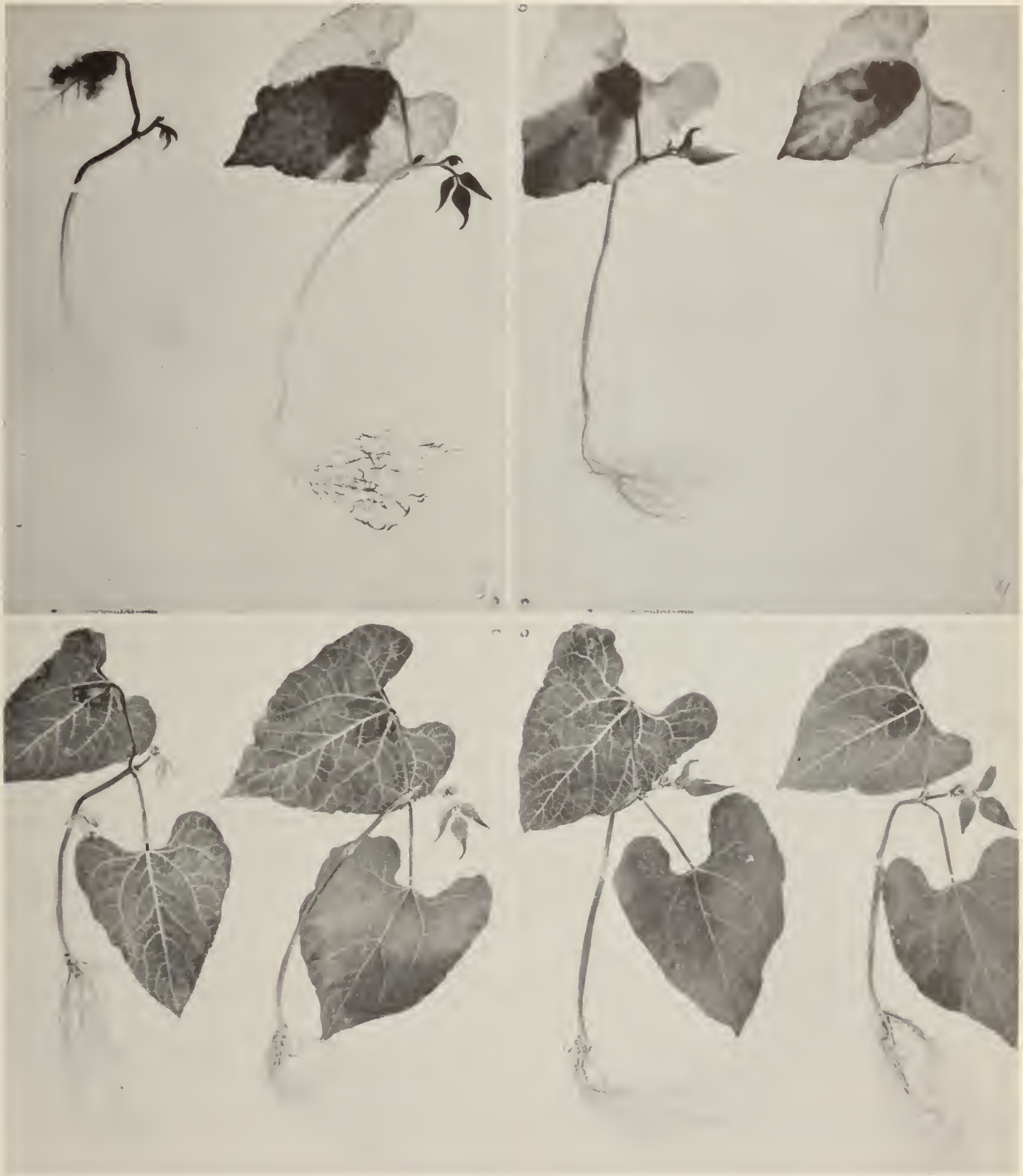


Fig. VIII, 2. Plants (below) and autoradiographs (above) of bean treated for 1 day with (left to right) 2,4-D*, amitrole*, MH*, and dalapon* The 2,4-D*-treated plant shows symplastic movement only; the other three display both symplastic and apoplastic movement.

of MH* and dalapon* in the 4-day trial with bean.

COMPARATIVE MOVEMENT OF 30 LABELED TRACERS IN BARLEY AND BEAN PLANTS

As it became apparent that each new labeled compound has a characteristic translocation pattern in plants, it seemed reasonable that a standardized test using autoradiography as the principal tool could be devised to give information concerning the pattern. Thus, a technique was designed using leaf and root application to barley and bean seedlings, with treatment times of 1, 4, and 16 days. Some twenty-seven organic compounds and three mineral elements have now been run through this test. The following paragraphs summarize the results; table VIII, 1. gives the distribution patterns in quantitative terms and figures VIII 5, 6, 7, illustrate the results of 16-day trials with 2,4-D*, MH*, amitrole*, dalapon* and Zn^{65}.

ALANAP:* In the leaf of bean, Alanap* moves in both the apoplast and symplast. Phloem movement to roots is weak and there is no circulation via the xylem.

In barley, uptake by the leaf is strong with medium phloem movement to roots and no recirculation.

Uptake by bean roots is strong and transport via xylem is medium with no redistribution.

Uptake by barley roots is low with some transport to the tops, but there is no accumulation.

AMIBEN:* Uptake and movement by bean leaf is symplastic, with medium-strong transport to roots and no retransport via the xylem.

In barley leaf, uptake is medium with appreciable movement to the roots.

Uptake by bean roots is strong with no movement to tops via the xylem.

Uptake by barley roots is medium, with only traces reaching the tops.

AMITROLE:* This compound enters the bean leaf readily and moves in both symplast and apoplast (fig. VIII, 2). Transport to roots and buds is strong, and there is no recirculation via xylem.

Barley leaves absorb this compound freely, and it moves freely to roots with a bit of recirculation.

Bean roots absorb amitrole* in moderate quantity and transport it to the tops in increasing amounts with time. It is retranslocated from mature leaves to the buds via phloem.

Barley roots absorb and transport amitrole* in moderate quantity; the amount moved increases with time.

AMMONIUM THIOCYANATE:* This compound penetrates the leaf of bean and moves in both apoplast and symplast. Phloem movement is limited to traces in epicotyl and hypocotyl in 4 and 16 days.

Barley leaves absorb and move this compound in apoplast and symplast, and it is transported to roots in low concentration.

Bean roots absorb and transport ammonium thiocyanate* through the stem to the leaves.

Barley roots also absorb and translocate this compound, but in small quantities.

ARSENATE As^{77} is a short-lived isotope, but we were able to obtain satisfactory autographs in coffee seedlings. As^{77} entered the mature leaf and moved symplastically in fair concentration through the stem and into the roots; absorbed by roots, it concentrated to a high level. It also ascended the stem in medium amount, and there was light but thorough labeling of the leaves. There was no evidence of redistribution via the phloem.

ATRAZINE:* Bean leaves absorb this compound and it moves freely in the apoplast but not at all in the symplast.

Barley leaves show only apoplastic movement of atrazine* from the region of application to the tips, with no symplastic movement.

Roots of bean absorb atrazine* and transport it to the top of the plant via xylem.

Roots of barley absorb and transport this compound in the same way. There is no evidence of retransport via phloem.

BARBAN:* This compound shows strong apoplastic movement in the bean leaf, no symplastic movement, and no redistribution.

Barley leaves absorb and translocate barban* in the apoplast, but only slightly in symplast.

Fig. VIII, 3. Plants and autoradiographs of bean treated for 1 day with (left to right) P^{32}, Zn^{65}, and Ca^{45}. P^{32} and Zn^{65} show symplastic movement only; Ca^{45} shows apoplastic movement only.

Bean roots absorb barban* in large amounts, but move it to the tops in small quantities only.

Barley roots absorb fair quantities of barban*, but transport only a small amount.

DACTHAL*: Bean leaves show little tendency to move this chemical via either symplast or apoplast; it is present in stem and roots in traces only.

Dacthal* is also only slightly mobile in barley leaves and is present in roots in traces only.

Roots of bean accumulate Dacthal* to high concentration, but move very little beyond the hypocotyl.

Roots of barley neither absorb nor translocate Dacthal* in large quantity.

DALAPON*: Bean leaves absorb and translocate dalapon* principally via symplast into the stem, but only traces stay in the roots (fig. VIII, 2, 4). Traces in

opposite leaves indicate migration from phloem to xylem and retransport via xylem.

Barley leaves absorb and move dalapon* rapidly, but this compound does not accumulate in roots.

Bean roots absorb dalapon* only in small quantities; this moves into the stem but reaches the leaves in traces only.

Barley roots likewise absorb and move dalapon* in small quantities.

2,4-D*: This compound is readily absorbed by bean leaves and distributed throughout the stem via the symplast. There is no movement in the apoplast and no retransport. Concentration in the roots is medium (fig. VIII, 1, 2, 5).

Barley leaves absorb and transport 2,4-D* readily via symplast, not via apoplast.

Bean roots absorb 2,4-D* to high concentration, but move little into the stem and none into the leaves.

Barley roots absorb much 2,4-D*, but transport little.

2,4-DB*: This compound is very immobile in bean. It labels the treated spot on the leaf, but scarcely moves beyond this.

In barley leaf, 2,4-DB* moves little beyond the treated leaf.

2,4-DB* is strongly absorbed by bean roots, but moves through the stem at very low concentration and labels the leaves very little.

Barley roots absorb 2,4-DB* strongly and move a little to the tops.

DURASET*: This compound penetrates the bean leaf and produces a pattern of apoplastic movement; only traces arrive in the stem and roots.

Barley leaves are heavily labeled near the treated spot, but only a small quantity of Duraset* reaches the roots.

Bean roots absorb Duraset*, but fail to move much to foliage.

Barley roots absorb Duraset*, and appreciable quantities reach the tops in 4 to 16 days.

EPTAM*: This compound readily penetrates the bean leaf; it moves acropetally along the veins (not via the apoplast). Basipetally, it moves only in traces, possibly because of loss in the vapor form.

Barley leaves show high concentration of Eptam* in the treated spots, and little moves to roots.

Absorbed by bean roots, Eptam* moves quite readily along the stem and into the leaves. There is no redistribution.

Barley roots absorb only limited quantities of Eptam*; it moves to leaves in small amounts only.

ETHYL, ETHYL-N-BUTYL THIOLCARBAMATE*: An analogue of Eptam, this compound is, if anything, less mobile in bean; it penetrates the leaf and moves acropetally in the veins, but moves to stem and roots in traces only.

In barley, this compound enters the leaf but scarcely moves beyond the treated spot.

This compound is absorbed in quantity by roots of bean and moves in fair amounts through the stem and into the leaves.

Barley roots accumulate only a small concentration of this compound, and it moves to the leaves in like quantities.

MALEIC HYDRAZIDE*: This is the most freely mobile compound that we have studied. It is absorbed by bean leaves and moves acropetally via the apoplast, basipetally via the symplast and phloem. It is present throughout stems and leaves and tends to recirculate, as shown by its presence in opposite leaves (figs. VIII, 2, 4).

MH* is also very mobile in barley. From the leaves it moves throughout tops and roots of treated plants, showing high concentration in young leaves.

Absorbed in medium quantities by bean roots, MH* moves through the stems and appears in leaves in low quantities.

Barley roots absorb medium quantities of MH*, but pass it to the foliage in traces only.

MONURON*: This compound enters the bean leaf readily and moves acropetally in the apoplast; it seems unable to enter the symplast and hence is missing from the rest of the plant.

Monuron* moves in the barley leaf only from the region of application to the tip; no basipetal movement takes place (fig. VI, 3).

Monuron* is readily absorbed and translocated by bean roots; with time, it builds up to high concentration throughout the plant.

Barley roots also absorb monuron*

Fig. VIII, 4. Bean plants and autoradiographs after treatments with MH* (left) and dalapon* (right). Treatment time 4 days. Leaves opposite treated ones show as a result of leakage of tracers from phloem to xylem. Dalapon fails to accumulate in old roots.

and transport it to leaves where it accumulates to high levels (fig. VII, 6).

PENTACHLOROPHENOL* Tested in bean only, this compound moves along the midrib and side veins from the treated spot toward the leaf tip; it shows no movement toward the main stem. In root application it accumulates to a medium concentration in the contacted roots, moves in only a light trace through the stem and petioles, and does not reach the leaves.

N-PROPYL, DI-N-PROPYLTHIOL CARBAMATE* Another Eptam analogue, this material is similar in distribution to the latter. In bean leaf it is absorbed and moved acropetally in the veins, basipetally its movement takes place only in traces.

Barley shows apoplastic movement of this compound in the treated leaf, but it moves to roots in traces only.

Bean roots absorb this compound to fairly high concentration but move it to foliage in moderate amounts only.

Barley roots absorb and move this compound in small quantities.

3-CHLOROPHENYL-ALPHA PROPIONIC ACID* In bean leaf, this compound is readily absorbed. It moves acropetally in veins, basipetally in the symplast, concentrates in the bud, and reaches the root in medium to large quantities.

In barley leaf, this compound moves both via the apoplast and the symplast; it goes into the other leaves and reaches the roots in low amounts.

Absorbed in medium quantities by bean roots, this compound moves through the stem and into the leaves in traces only.

Barley roots absorb only small amounts of this compound and move it in traces.

PROPYL, ETHYL-N-BUTYLTHIOL CARBAMATE* This third analogue of Eptam is promising in field trials. Absorbed and moved acropetally in veins of bean leaf, it is translocated in small to trace amounts.

In barley, this compound moves in the apoplast, but scarcely enters and moves in the symplast. The roots were almost devoid of label.

In bean roots this compound is absorbed to high concentration, and it moves in medium amount through stem and leaves.

In barley likewise, it is taken up and moved to foliage in medium amounts.

SIMAZINE* The bean leaf absorbs simazine* and moves it acropetally in the apoplast; no simazine* enters or moves in the symplast.

The barley leaf likewise absorbs and moves simazine* via apoplast, but not via symplast.

Applied to bean roots, simazine* enters and moves readily throughout the stem and leaves.

In barley also, simazine* enters roots and moves freely to foliage. There is no sign of recirculation, as shown in figure VIII, 1.

SODIUM ACETATE* This metabolite is readily absorbed by the bean leaf and it moves acropetally in veins, basipetally in the symplast; here, it traverses the stem and reaches the roots in fair quantity. It does not transfer to xylem nor appear in the opposite leaf.

In barley, sodium acetate* enters and moves freely throughout the foliage and roots, where it may occur in high concentration.

This compound is absorbed strongly by bean roots, moves lightly into stems, and fails to reach the leaves.

In barley it is strongly absorbed by roots, and reaches the leaves only in traces.

SODIUM BENZOATE* This compound enters the bean leaf and traverses the lamina in the veins; it moves to stems in low amounts and reaches roots in low concentration.

In barley leaves, sodium benzoate* is strongly absorbed. It reaches the other leaves in low concentration, and appears in the roots in medium amounts.

In bean roots it is strongly absorbed; it moves in stems in traces, but fails to reach the leaves.

In barley it builds to high concentration in roots, but reaches the leaves in small quantities only (fig. VII, 5).

2,4,5-T* This compound shows only symplastic movement in the bean leaf. It moves in moderate amount in the stems, but reaches the roots in low concentration.

In barley, this compound is high in concentration in the treated leaf, but reaches the roots in traces only.

In bean roots, 2,4,5-T* enters in medium amount, moves in small amount, and fails to reach the leaves.

In barley, 2,4,5-T* is absorbed slowly with time and builds to medium concentration in 16 days; however, it reaches the foliage only in traces from root absorption.

2,4,5-T BUTOXYETHANOL ESTER* This heavy-ester compound of 2,4,5-T* is absorbed slowly. It builds to high concentration in the treated leaf where it moves acropetally in the leaf veins and basipetally in the symplast. It reaches the stem in medium to high quantities and the roots in medium quantities.

In barley it moves both acropetally and basipetally in the treated leaf. It enters the other leaves, particularly the young ones, and reaches the root in small to medium quantities.

In bean, the compound enters roots in moderate amount, but moves in the stem in traces only and fails to reach the leaves.

Fig. VIII, 5. Autoradiographs of bean plants after 16-day treatments with 2,4-D* (right) and amitrole* (left). The amitrole treatment shows redistribution of the tracer from old to young leaves as the plant grows.

Fig. VIII, 6. Autoradiographs and plant mounts after 16-day treatments with MH* and dalapon*. Redistribution is prominent; leakage is shown by the labeling of the opposite primary leaf.

In barley root, absorption is medium; the compound moves to leaves in low to trace amounts.

TRICHLOROPROPIONIC ACID* This compound enters the bean leaf readily, translocates acropetally in the veins and basipetally in the symplast. It moves through the stem and into the roots in medium to small quantities.

In barley it moves in both directions in the treated leaf, infiltrates the untreated leaves, and moves into roots in medium quantities.

In bean roots it builds to high concentration, but moves in stems in small to trace amounts; it fails to reach leaves in 1 and 4 days, and reaches them in traces only in 16 days.

In barley roots it accumulates to medium quantities and moves to tops in small quantities.

INDOLE-3-ACETIC ACID* In barley, IAA* enters the leaves more freely than 2,4-D* and moves into roots at low concentration from leaf treatment. Applied to roots it is strongly accumulated and moves into tops in medium concentration. It has no tendency to migrate from phloem to xylem - at least not in a mobile form. Figure VIII, 1, shows the 1- and 16-day results in barley.

PHOSPHORUS P^{32} enters the leaf rapidly, moves symplastically through petiole, epicotyl and hypocotyl, and concentrates in the terminal bud and in root tips (figs. VII, 7; VIII, 3).

CALCIUM Ca^{45} enters the leaf in large amounts and moves strongly in the apoplast; it moves scarcely at all in the symplast. It does not reach the petiole or stem, and it stays in the same location for 16 days or more (figs. VII,7; VIII,3).

ZINC Zn^{65} enters the leaf rapidly, does not move in the apoplast, moves about like P^{32} in the symplast, and accumulates in the terminal bud and root tips (figs. VIII, 3, 7). It retranslocates freely, moving into young leaves as they grow; in the stem it attains highest concentra-

Table VIII, 1
Translocation Patterns of Labeled Tracers in Bean Plants
(Results are averaged, hence not specific for time.)

Compound	Leaf application, translocation						Root application, translocation			
	In the leaf, via:			In bud	In stem	In roots	Sorption by roots	Transport to tops		
	Veins	Apoplast	Symplast					Hypocotyl	Epicotyl	Leaves
Alanap	+	++	tr	tr	tr	tr	++	+	+	+
Amiben	++	o	+	+	++	+	++	o	o	o
Amitrole	o	+++	++	+++	++	+	++	+	+	++
Ammonium thiocyanate	++	o	tr	tr	tr	o	++	+	+	++
Arsenate†	o	o	++	++	+	+	+++	++	++	+
Atrazine	+	+++	o	o	o	o	++	+	+	++
Barban	+	+++	o	o	o	o	+++	+	+	+
Dacthal	tr	o	tr	tr	tr	tr	++	+	+	tr
Dalapon	+	+	++	++	++	tr	+	++	+	tr
2,4-D	+	o	++	+++	+++	+++	++	+++	tr	o
2,4-DB	+	o	o	o	o	o	+++	+	+	+
Duraset	o	++	tr	+	tr	tr	++	+	+	+
Eptam	++	+	o	tr	tr	tr	++	++	++	++
2060	++	o	tr	tr	tr	tr	+++	++	++	+
IAA‡	+	+	++	-	-	++	+++	-	-	+
Maleic hydrazide	o	++	++	++	++	++	++	+	+	tr
Monuron	o	++	o	o	o	o	++	++	++	+++
PCP	tr	o	o	o	tr	tr	++	tr	tr	o
1607	++	+	tr	tr	tr	tr	++	++	++	+
3-CP§	++	+	++	+++	++	++	++	tr	tr	o
2061	++	o	tr	tr	tr	tr	+++	+++	+++	++
Simazine	o	++	o	o	o	o	++	+	+	++
Sodium acetate	+	o	+	+	+	+	+++	tr	tr	o
Sodium benzoate	++	o	+	+	++	+	+++	tr	tr	o
2,4,5-T	o	o	++	++	++	+	++	tr	tr	o
2,4,5-TB ester	+	o	++	+++	++	+	++	tr	tr	o
TCP ¶	+	+	+	+	+	+	++	tr	tr	o
Ca^{45} ‖	++	+++	o	o	o	o	-	-	-	-
P^{32} ‖	o	o	+++	+++	+++	++	-	-	-	-
Zn^{65} ‖	tr	o	++	+++	++	+	-	-	-	-

Key to symbols: tr = a trace; + = light gray; ++ = dark gray; +++ = black; - = no data.

† Coffee
‡ Barley
§ 3-chlorophenoxy-alpha-propionic acid
¶ Trichloropropionic acid
‖ Leaf treatment only, bean

Fig. VIII, 7. Redistribution pattern in bean and barley treated for 16 days with Zn^{65}.

tion in the epicotylar node. It continues to move into the plant and after 16 days the roots of bean were heavily labeled with dense concentrations in root tips.

In order to bring the important results of this study together in visual form, table VIII, 1, has been compiled to allow a quick comparison of the major differences.

There are many mechanisms of herbicidal action, and different and distinct patterns of distribution are desirable in different compounds. To control perennial weeds one may use a highly mobile herbicide (amitrole, for example) as a translocated spray in areas where valuable crops are growing. On the other hand, on roadsides, ditchbanks and other noncropped agricultural and industrial areas it is useful to have compounds of low water-solubility (urea and triazine herbicides, for example) which are readily absorbed from the soil.

In cropped areas, residues are always a potential problem. For pre- and post-emergence soil application, compounds such as amiben, 3-CP, 2,4,5-T and TCP, IPC and CIPC, which do not translocate from roots to tops, can be used on a no-residue basis. Others, such as the triazines that are readily detoxified by certain crop plants, may be used if their decomposition products are harmless. The advantage of the chlorophenoxy compounds as pre-emergence materials is their strong contact action against emerging seedlings and the fact that they concentrate in roots of larger plants and move to tops in minimal amounts only. Even the amounts shown as images in autographs may be largely converted to innocuous compounds that do not appear in residue analyses. Thus, it is obvious that information gained from autoradiographic studies must be interpreted in view of the many mechanisms of herbicidal action and in terms of residue problems. For example, autographs of the herbicide Dacthal used by root application proved useful in registering it on a no-residue basis.

Chapter IX. Effects of Time and Dosage on Transport

TIME RELATIONS

When using tracers in autoradiography the time-dosage relations must be determined for each isotope. An early experiment with C^{14}-labeled 2,4-D involved a dosage series including 1, 5, 10, 20 and 50 µg of radioactive 2,4-D having a specific activity of 1.24 mc per mmole with a treatment time of 2 hours. This test proved that 5 µg or more of this formulation was required to produce a satisfactory autograph. This amount is 0.028 µc, a quantity just sufficiently radioactive to provide labeling of a bean or barley plant of a size to fit a 10 x 12-inch X-ray film. We now use 0.10 µc as a standard dosage for survey work with new chemicals.

A comprehensive time series was next conducted, using an arithmetical series from 1/4 to 144 hours (Crafts, 1956*a*). Because of the killing and drying methods used, autographs showed the artifact of xylem movement (Crafts, 1956*a*, figs. 10 through 14). By correlating stem bending with tracer distribution they did prove however that approximately 3 hours were required for absorption and movement into roots from the primary leaf of bean; about 6 hours were required for transport into apical buds.

Since these early tests, many experiments have included a series of exposure times so that a clear picture of the time relations of tracer uptake could be obtained. The tests described in this chapter include 1-, 4-, and 16-day treatment times designed to provide evidence on initial uptake and rapid distribution (1 day), continued uptake and distribution (4 days), and pattern of redistribution in time (16 days).

Figure IX, 1, shows a time series involving MH* treatments on barley leaves for periods of 3, 9, and 27 hours. Placed on leaf 2 of these plants, MH* moved into roots quite rapidly but into tops more slowly. The droplets of formulated MH*, containing 0.05 uc per treatment in 50 per cent ethyl alcohol and 0.1 per cent Tween 20, dried in about 30 minutes; movement into the leaf and around the plant continued for hours. Drying of the droplets evidently does not stop the uptake of applied molecules, and their ultimate destination depends upon their mobility through the living symplast.

These tests prove that tracers formulated with surfactant may continue to move through cuticle and into living leaves for days after application. This is particularly well illustrated in figure IX, 2, which shows zebrina treated with 1.0 µc per leaf for 1 and 20 days. Applications were made to the upper surfaces of hypostomatous leaves, and the slow continued uptake of 2,4-D* proves that these molecules may traverse the cuticle and move through leaf, stem, and roots for prolonged periods, providing they are formulated so as to contact the leaf surface from a liquid film.

THE EFFECTS OF DOSAGE UPON TRANSLOCATION

Early plot work with 2,4-D indicated that there was a dosage optimum with this herbicide when used as a systemic toxicant on perennial weeds. When our autoradiographic studies proved that 2,4-D*, and other systemic herbicides, move through the plant in the assimilate stream with food materials, it was postulated that high dosage, resulting in rapid-contact injury, might knock out the phloem-

Fig IX, 1. Transport of MH* in barley after 3, 9, and 27 hours; treatment on leaf 2. Dosage was 0.05 µc; specific activity 0.5 mc per mmole. Droplets of treating solutions were dry after 30 minutes.

transport system and hence prevent systemic distribution. In order to check this hypothesis, a dosage-series experiment was designed to explore a range of dosages covering all normal application rates for seven herbicides.

The dosage series used was 0.02 µmole, 0.05 µmole, 0.1 µmole, 0.2 µmole, 0.4 µmole and 0.8 µmole per application applied to one primary leaf of bean. The same amount of chemical dissolved in 100 ml of culture solution was used to treat root systems of individual culture solution plants. The results of this experiment were interpreted as follows.

2,4-D* All dosages caused stunting and deformation of the plants. The labeling in the hypocotyls increased from 0.02 µmole through 0.1 µmole and then decreased. It was assumed then that dosage of 2,4-D on bean should not exceed this amount for optimum results. The root treatment showed a regular increase in concentration with dosage, and a consistent reduction in size of the trifoliate leaves that developed during the treatment. Swollen tips of treated roots had high labeling; there was little accumulation in the hypocotyls. Subsequent treatments at 10 µmole per 20 ml culture solution per plant resulted in increasing transport from roots to tops (figs. VII, 3, 4). This last dosage is roughly equivalent to the actual absorption of around 2 pounds of 2,4-D acid per acre of bean plants - a dosage which would be used only to control a perennial weed by soil application. It is considerably above the dosage range used in pre-emergence applications for controlling seedlings of annual weeds.

Fig. IX, 2. Translocation of 2,4-D* in zebrina after 1 day (left) and after 20 days (right). Tracer from this formulation in 50 per cent alcohol and 0.1 per cent Tween 20 apparently continued to move into the plants long after drying of treatment solution on leaves.

DALAPON*: This compound showed a consistent movement from treated leaf to bud and expanding trifoliate leaf, increasing with increasing dosage. No optimum was observed. Dalapon* moved through the roots into hypocotyls and terminal buds to high levels. Concentration was high in root tips, but only medium in older roots.

MONURON*: This compound showed strong apoplastic movement in bean leaves, the intensity of labeling increasing with dosage. There was no symplastic movement into the stem and roots; a slight trace appeared in trifoliate leaves and buds at dosages of 0.4 and 0.8 μmole. Monuron* enters roots and moves in the transpiration stream very readily. At 0.02, 0.05 and 0.1 μmole dosages, the primary leaves showed more labeling than the trifoliates; at the higher dosage rates all leaves were black. The shoots were much inhibited through the dosage range of 0.2 to 0.8 μmole, following the series in intensity. Concentrations in roots were medium to high through

the series; in hypocotyls, low to high. Figure IX, 3, shows the results of this treatment for 1 day; figure IX, 4, shows the 4-day results.

AMITROLE* Both symplastic and apoplastic movement occurred in bean with amitrole*. Concentrations were high in buds at all dosage levels; there was no growth inhibition. Concentrations were high in root tips at all levels; opposite primary leaves had light labeling at dosages of 0.2, 0.4 and 0.8 µmole.

Amitrole* applied to roots showed a graded series of concentrations with dosage ranging from medium to high; in hypocotyls, the range was from low to high There was no inhibition of shoot growth, and buds showed accumulation to higher levels than subtending internodes.

2,4,5-T* This compound had little tendency to move in the apoplast, and its symplastic movement was less than that of 2,4-D*. Inhibition of buds and trifoliate leaf growth followed the dosage level, as did tracer concentration in the buds. As with 2,4-D*, the level of tracer in roots from leaf treatment was low, and was independent of dosage; hypocotylar concentrations followed the series, but were not high at any level.

In root uptake, 2,4,5-T* concentrations in roots and amounts translocated to tops follow the dosage series. Movement up the hypocotyl increased with dosage and both primary leaves carried the label at 0.8 µmole dosage.

MALEIC HYDRAZIDE* This compound is very mobile in plants, and distribution patterns in leaves are both apoplastic and symplastic. MH* concentrates in the young leaves and buds and varies from light to

Fig. IX, 3. Autoradiographs showing results of treating bean plants with a dosage series of monuron for 1 day. Dosages, left top to right bottom, 0.02, 0.05, 0.1, 0.2, 0.4, and 0.8 µmole; there is increased uptake with increased dosage.

Fig. IX, 4. Autoradiographs showing results of treatments as in fig. IX, 3, but left for 4 days. The higher dosages inhibited growth from the second day until the fourth.

medium in the hypocotyls. MH* was present in the roots from light to medium through the dosage series and some reached the opposite leaves, indicating transfer from phloem to xylem.

MH* enters roots and moves upward in the transpiration stream.

Concentration in the primary leaves increased through the dosage series, being higher in the young leaves and buds than in stem and primary leaves. This indicates symplastic movement from the primary leaves after arrival in the transpiration stream.

In conclusion, only 2,4-D showed an optimum dosage; 2,4,5-T penetration limits the amount of this chemical that enters the symplast. Monuron does not enter the symplast. Amitrole penetrates slowly and translocates fast and apparently does not build up to a toxic concentration. The same relation may explain the lack of an optimum for dalapon and MH. Solubility, penetration rate, speed of transport, inherent toxicity, and accumulation in the symplast all enter into this problem. Within the range of dosages and the series of compounds used, 2,4-D seems to be the only herbicide that causes acute injury to the transoprt system..

Chapter X. Interaction of Herbicidal Molecules

SYNERGISM, ANTAGONISM, INTERACTION

The terms "synergism" and "antagonism" are often used in reports and discussions of herbicidal action. These terms, while convenient, have little basic meaning in terms of chemical mechanism; we choose to use the term "interaction" to express both concepts, and we realize the need to study such responses by chemical and physiological means.

In studies on large zebrina plants growing with their roots spread on filter paper saturated with culture solution (fig. II, 2), it was shown that amitrole* moved throughout the stem and root system and accumulated in root tips in a 4-day treatment period (fig. X, 1). Labeled sucrose formed from the $C^{14}O_2$ from urea* showed a similar distribution (fig. X, 2). On the other hand, labeled 2,4-D moved into the upper portion of the root system but did not reach the lower growing regions (fig. X, 3). The question naturally arises as to whether this failure to move is the result of accumulation of 2,4-D* along the route of transport, or whether it is caused by some physiological effect on the phloem. To answer this question 2,4-D* was applied to similar zebrina plants, and 4 days later amitrole* was applied to some plants, while urea* was applied to others. In all cases the autographs produced were similar to the original 2,4-D* autographs: they showed little or no tracer in the root tips (fig. X, 4). This indicated that 2,4-D* was having an effect on the phloem and was inhibiting distribution of the amitrole* and the sucrose* from urea*.

EFFECT OF 2,4-D ON PHLOEM

Because application of 2,4-D before application of amitrole* inhibited distribution of the latter in zebrina, it was decided to explore the dosages and pretreatment times that effectively block the phloem. Three experiments were conducted: one in which 2,4-D and amitrole* were applied to one primary leaf of bean, one in which 2,4-D was applied to one primary leaf and amitrole* applied to the opposite leaf, and one in which 2,4-D was applied to roots and amitrole* to one primary leaf. Dosage was varied through the series - 1/100 μmole, 1/10 μmole and 1 μmole. Each experiment in which leaf application was made involved application of both chemicals at the same time, application of 2,4-D 24 hours before the amitrole* and application of 2,4-D 48 hours before the amitrole*.

The most obvious response followed application of amitrole* 48 hours after the 1.0 μmole dosage of 2,4-D. In this case the amitrole* was limited to the bud, primary leaves, and epicotyl; none reached the hypocotyl and roots (fig. X, 5). The 1-day interval at 1.0 μmole dosage showed a slightly lower transport of amitrole* to the roots; all other dosages and time intervals showed no effect. Inhibition was greater where both applications were made to the same leaf, and the there were no differences where roots were treated.

This experiment suggests that only when dosage is high, and only when an appreciable time interval is left between application of 2,4-D and application of a second tracer, is there inhibition of phloem transport of the second tracer. These tests also prove that 2,4-D will inhibit translocation of a separate tracer

Fig. X, 1. Translocation of amitrole* in zebrina over a 4-day treatment time. The tracer has undergone a thorough distribution by symplastic movement (for method of culture see fig. II, 2).

Fig. X, 2. Translocation of C^{14} as sucrose* derived from urea* in zebrina over a 4-day period. All active sinks are labeled including root hairs (see fig III, 2).

applied after the 2,4-D has had time to act. Presumably not only the movement of a second tracer, but movement of the 2,4-D and of foods and all other constituents of the assimilate stream is blocked. This may well be a key to the toxic action of 2,4-D on whole plants.

Eames (1949, 1950) found that 2,4-D caused phloem collapse in nutsedge and bean, and Muni (1959) showed that 2,4-D applied in lethal concentration to both susceptible and resistant species of monocotyledonous and dicotyledonous plants caused tissue proliferation, formation of galls and adventitious roots, and crushing of the phloem. Continued action also resulted in plugging of the xylem and complete disorganization of the vascular tissues.

In the zebrina plants, stoppage of transport occurred in the more mature vascular tissues of the stem and older roots. This is a somewhat different case than those studied by Muni, as the action of 2,4-D here is more subtle. The researcher may well ask if there is a key to this puzzle in the known physiology of phloem.

Anatomical (Esau, 1953) and physiological (Crafts, 1961*b*) work on phloem indicates that the sieve tubes (the specialized conduits through which rapid transport takes place) are highly specialized elements (Esau and Cheadle, 1961). Starting much like normal young parenchyma cells, they soon go through a series of characteristic and unique developmental changes and become longitudinally permeable conduits through which assimilates apparently move in a stream. This highly permeable functional period may last for only a short time in the case of protophloem sieve tubes, longer in the case of metaphloem elements, and even longer for secondary sieve tubes - possibly through a single, or, in a few cases, two annual functioning cycles. Eventually, however, every sieve tube enters a period of reduced functional activity, dies, and becomes obliterated, that is, crushed, or at least nonfunctional. This period of senescence may start after a few days, a few weeks, or many months of functioning, and it represents the final stage of maturation. Usually, senescence of sieve tubes is characterized by callosing of the sieve plates, increased thickening, and constriction of the sieve-plate protoplasmic connections.

Recent studies with the electron microscope indicate the possibility, long questioned by plant physiologists (Crafts, 1961*b*), that the protoplasmic connections of sieve plates actually constitute intervacuolar tubules (Esau and Cheadle, 1961). If this proves true for many species, the accretion of callose in the form of cylinders around these sieve-plate connections may very well constitute the mechanism by which sieve tubes are gradually closed off as they become obliterated. And when subject to injury by cutting of the phloem, by virus invasion, or by use of heat or inhibitors, this constriction of the sieve-plate connections may be the means by which phloem exudation is rapidly checked and by which translocation is hindered and stopped.

J. L. Key (1962) found that high inhibitory concentrations of 2,4-D decrease the ascorbate activity of cells and cause a shift in the oxidation state, and that low stimulatory concentrations cause increase in ascorbate activity and an opposite shift in the oxidation state (chart X, 1; Crafts, 1963). It is possible that this shift in the oxidation-reduction state of the phloem tissues may trigger the reaction that results in a hastening of sieve-tube maturation and obliteration. This would explain both the subtle decrease in transport capacity brought about by intermediate concentrations of 2,4-D in phloem, and the more obvious tissue proliferation that causes crushing and death in the younger roots brought about by high amounts of 2,4-D. Again, it becomes evident why the exact physiological state of a plant is so important in determining the extent of damage that results from 2,4-D treatment - and the capacity of shoot buds from roots and rhizomes for vegetative reproduction takes on added significance with respect to the individual susceptibility of weed species.

2,4-D PRETREATMENT AND INHIBITION OF AMITROLE-C^{14} TRANSLOCATION

In our autoradiographic translocation studies, 2,4-D always lagged behind amitrole in extent or distance of distribu-

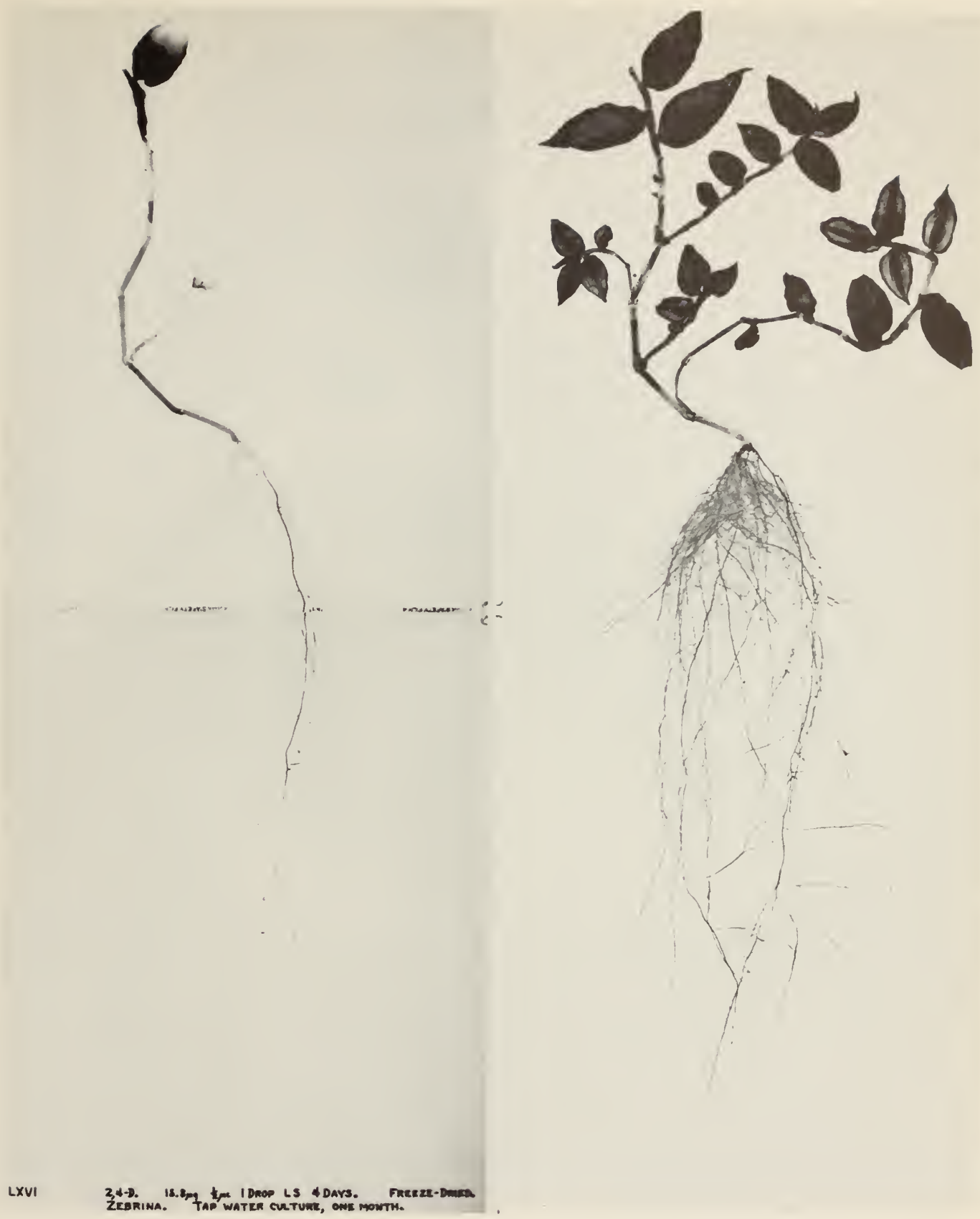


Fig. X, 3. Restricted distribution of 2,4-D* in zebrina in 4 days. Many roots are unlabeled, and some are labeled in their more mature regions; root tips and root hairs are free of C^{14}.

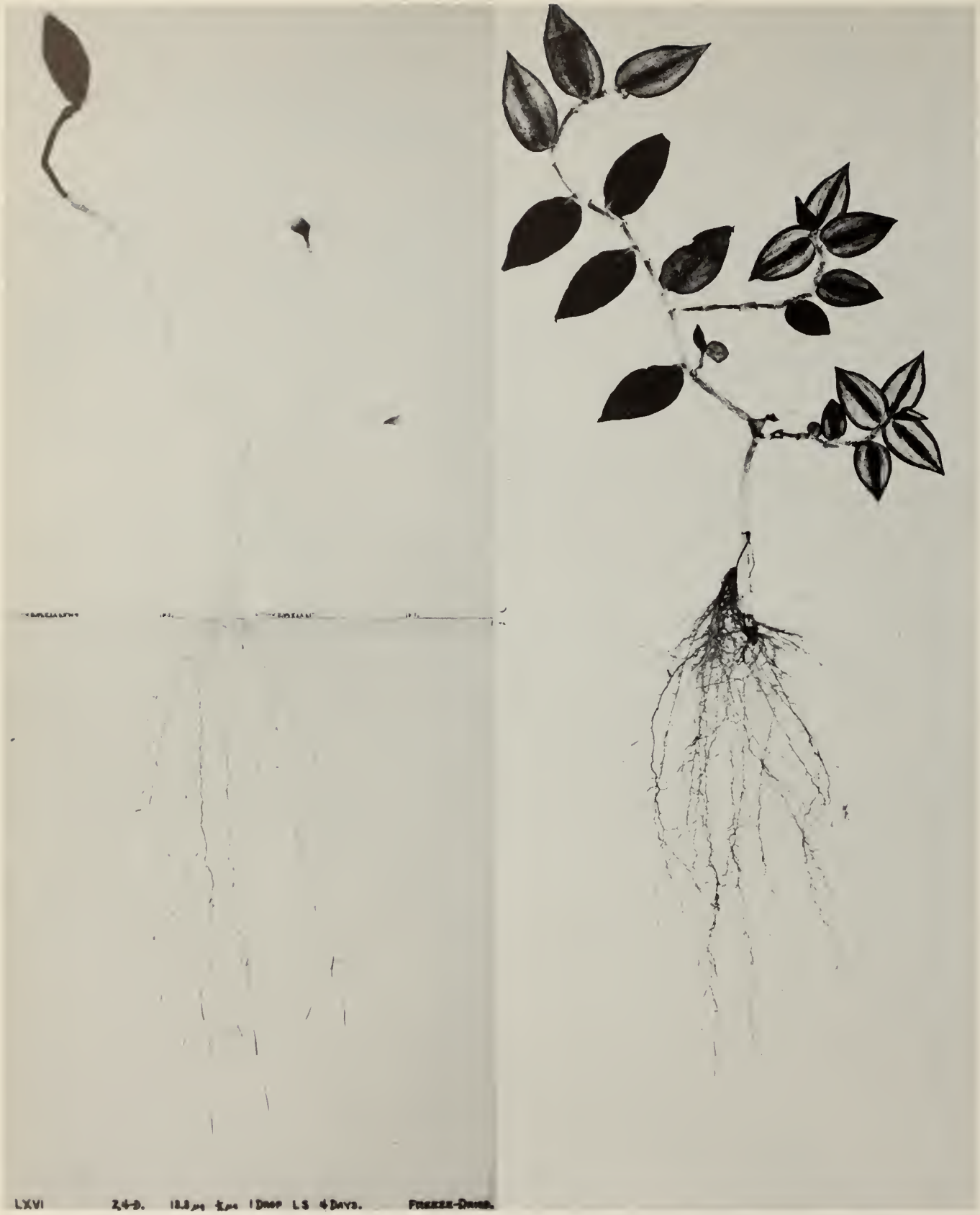


Fig. X, 4. Restricted distribution of amitrole* applied 4 days after a 2,4-D* application. Some roots are unlabeled and labeling is weak in some; root hairs lack C14.

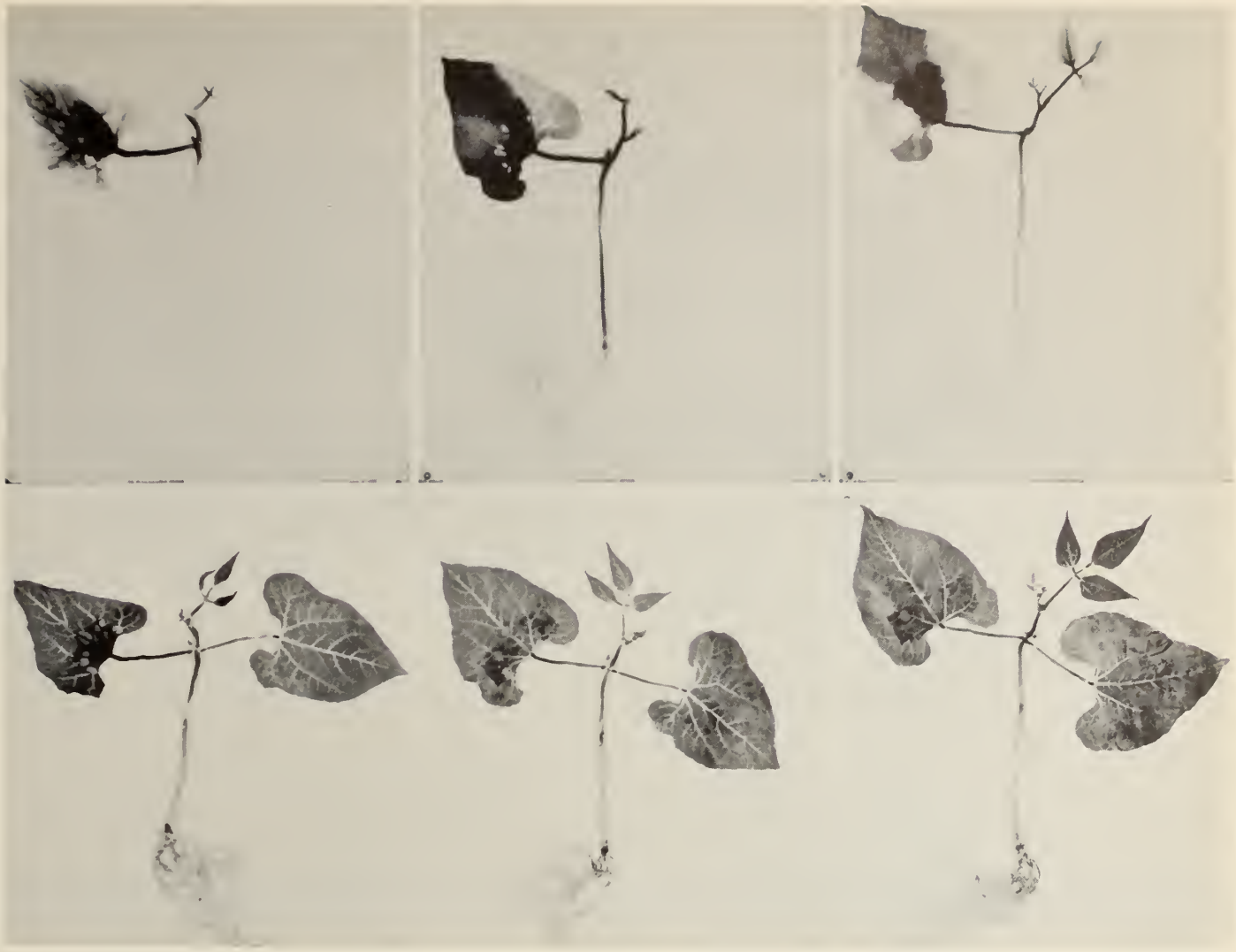


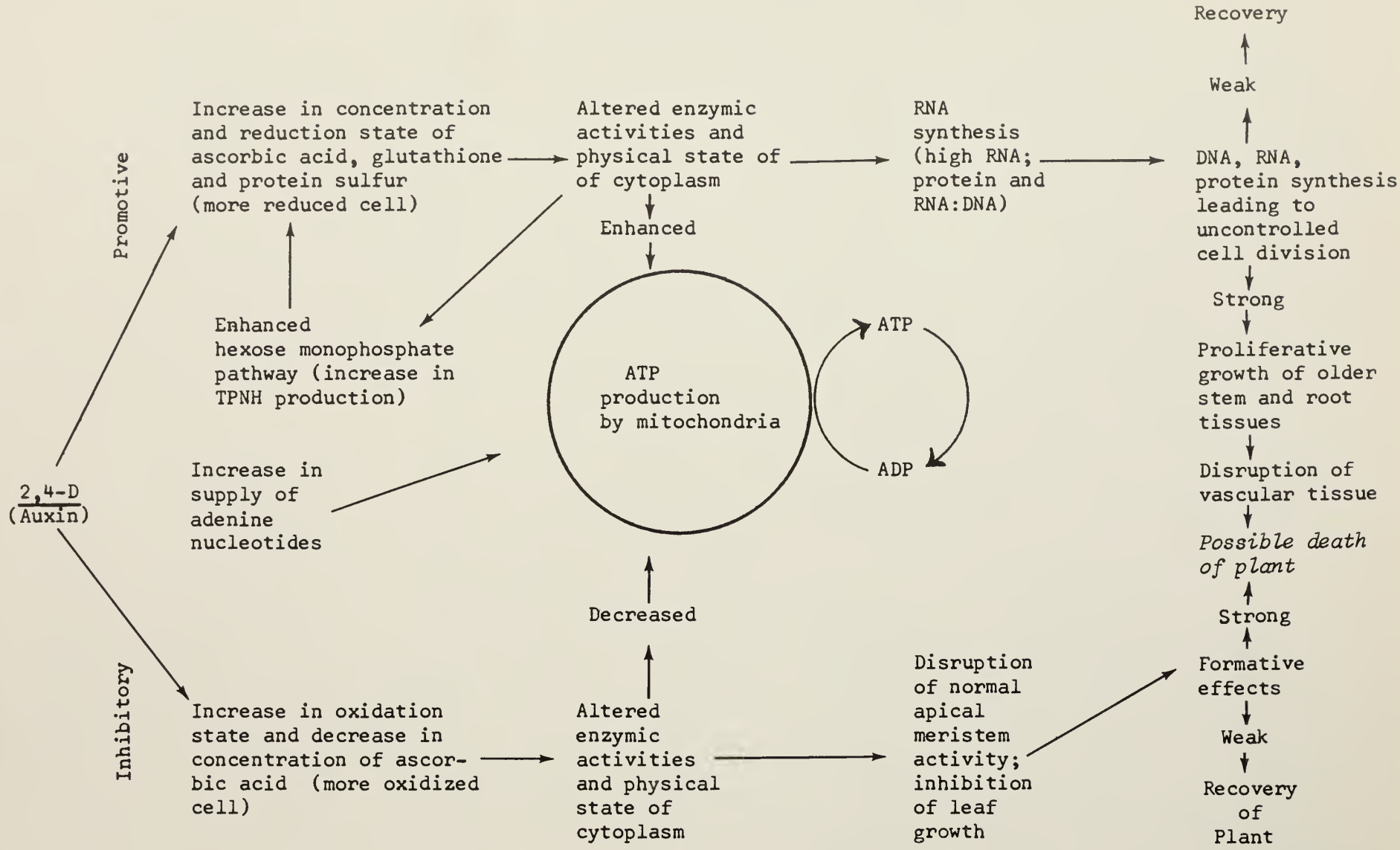
Fig. X, 5. Results of 2,4-D application on translocation of amitrole*. Left, 2,4-D applied 2 days before the amitrole*; center, 1 day before; and right, 2,4-D and amitrole* applied simultaneously.

tion, regardless of plant species. Lateral movement and absorption by the tissues surrounding the vascular tissue were thought to be the cause.

To investigate this, it was decided to pretreat with 2,4-D and to compare 24-hour distribution patterns of amitrole-C^{14} as affected by these pretreatments. Dosages of 1/100, 1/10 and 1.0 micromole of 2,4-D acid at 2210 ppm (1/100 micromole per microliter) in 50 per cent ethanolic solution were used for pretreatments of 0, 1, and 2 days on a primary bean leaf or through the culture solution of 100 ml in pint jars. One-tenth per cent of Tween 20 was incorporated in 2,4-D and amitrole* solutions, and 27 bean plants in primary leaf stage were used.

The lowest dosage applied to the leaf in no way affected the distribution of amitrole* of 1-day treatment time. With higher dosage the simultaneous application of 2,4-D with amitrole*, whether to the same or the opposite leaves, resulted in a distribution essentially unaltered because of 2,4-D. But with 1- and 2-day pretreatments with 2,4-D on one leaf, and with amitrole-C^{14} treatment on the same or the other leaf, this distribution pattern showed much less movement to the major sinks, the root tips and the stem tip. Also, the stem traced much darker than in the case of amitrole translocation alone because the amitrole* penetrated the cortex and epidermis; seemingly, the total amount of amitrole-C^{14} translocated was not much altered by 2,4-D pretreatment of either of the paired leaves, although the distribution pattern was altered (fig. X, 5).

Chart X, 1
Mode of Action of 2,4-D



In addition to phloem plugging a point of interest in this experiment is that a 24-hour pretreatment with 1.0 mmole of 2,4-D per plant rendered the phloem unable to retain amitrole. It is evident from the distribution pattern that one effect was on the permeability of the sieve tubes rather than on increased absorption by the surrounding tissues. This leakage along the route of translocation is probably very important, particularly when the movement is downward, because the leaking tracer would be translocated upward in the xylem.

Application of 2,4-D via the culture solution, even at 2.2 ppm, did not stop amitrole-C^{14} translocation from the leaf to the root tips, though the growth of the bud was nearly completely halted and the plant was slightly wilted. From other experiments it is known that the inhibition of the bud growth in this case is not because of the presence of 2,4-D; there is no 2,4-D in the bud.

In a similar experiment with zebrina plants cultured in pint jars, it was shown that a 2,4-D dosage of 1.0 µmole, spot-applied to a leaf 1 or 2 days prior to amitrole-C^{14} application to the leaf above, produced similar results; total movement of amitrole-C^{14} (4-day treatment time) out of the treated leaf was not much altered. The distance of translocation was reduced to an internode or two. The leakage of the amitrole-C^{14} from the phloem into the other tissues of the stem greatly intensified the images of these internodes adjacent to the treated leaf. Additionally, leakage finding its way into xylem was carried into a mature leaf.

From these 2,4-D pretreatment experiments it is evident that tissue response to 2,4-D of sublethal dosage is a part of the limiting factor in 2,4-D translocation and distribution. In fact, phloem failure from 2,4-D seems to be intimately related to the mode of action of this chemical when used as a herbicide.

Chapter XI. Symplastic and Apoplastic Movement

MECHANICS OF ABSORPTION

In the early chemical and physiological studies on herbicides it was suggested (Crafts and Reiber, 1945) that absorption of the dinitro weed-killers takes place via the lipoid phase of the cuticle, and that buffering the spray formulation of such a chemical would enhance uptake by presenting the toxicant molecules in the undissociated state for movement across this barrier. Because the undissociated parent acid molecules are more lipoid-soluble than the anions, they should diffuse across cuticle more rapidly. This idea was later applied to 2,4-D and other anionic toxicants (Crafts, 1948, 1956*a*).

Table XI, 1 presents data on absorption and translocation of 2,4-D in black-eyed pea plants following application to one unifoliolate leaf; variables are the pH of the applied solutions and the time in hours; values given are stem-bending in degrees.

When MH became available as a plant growth inhibitor it was used in a number of experiments and proved to be an active grass killer (Currier and Crafts, 1950). However, later studies (Smith, *et al.*, 1959; Crafts, Currier and Drever, 1958) proved that the mode of chemical activation used for dinitro compounds and 2,4-D did not apply in the case of MH; here, the only effective means of enhancing uptake was application in a saturated atmosphere.

Table XI, 2, presents data relating growth of Bermudagrass cultures to pH of applied MH solutions. It is obvious here that the optimum toxicity lies in the region of pH 6-8, with the greatest inhibition at pH 7.

The fact that high relative humidity might bring about a condition in the plant leaf in which the water continuum of the plant extends all the way to the leaf surface led to the postulation of an alternative aqueous route for solute uptake by plant leaves (Crafts, 1961*a*, *b*, *c*); such a mechanism obviously must involve the apoplast of the treated leaf.

When amitrole* was first applied to bean leaves a characteristic wedge-shaped pattern of labeling became evident (Yamaguchi and Crafts, 1958), and after considerable study our interpretation was that compounds causing this pattern were entering the leaf via the aqueous route and were moving in the apoplast along with the transpiration stream. Strugger (1938) had observed this type of movement of fluorescent dyes.

These patterns of apoplastic movement are brought about by amitrole* (fig. VIII, 2), MH* (fig. III, 4, and VIII, 2), atrazine*, monuron*, barban*, alanap* and Ca^{45} (fig. VIII, 3); even 2,4-D may cause this pattern of distribution in the cotyledon of cotton. Some of the above compounds are phloem mobile (amitrole*, MH*, alanap*, 2,4-d*) while others are not (monuron*, simazine*, barban*, Ca^{45}). Whether or not a compound will assume this wedge-shaped pattern of distribution seems to depend upon the avidity with which it is absorbed into the symplast, as contrasted with the rate of flow of the transpiration water in the apoplast. Such apoplastic distribution is of little consequence in the practical use of herbicides, but it has been extremely important in the interpretation of autoradiographs and in our understanding of the details of the process of absorption of

TABLE XI, 1
Absorption and Translocation of 2,4-D in Black-eyed Peas
Treated by Application to One Unifoliolate Leaf

pH of solution	Stem-bending (in degrees) at end of:								
	1 hr	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs	7 hrs	8 hrs	24 hrs
0.5	0	0	1.0	3.0	1.5	2.5	7.0	10.0	11.0
1.0	0	0	2.0	2.5	1.5	1.5	4.5	5.5	7.0
2.0	0	0	5.5	21.5	65.1	100.5	102.5	94.0	91.5
3.0	0	0	4.5	27.0	58.0	88.5	94.0	87.5	92.5
4.0	0	0	0.0	15.0	54.5	94.5	96.0	81.0	84.5
5.0	0	0	0.0	11.0	46.0	87.5	100.0	88.0	86.0
6.0	0	0	1.5	12.0	44.5	90.5	100.0	91.0	79.5
7.0	0	0	0.0	1.0	9.0	32.0	70.5	56.5	82.5
8.0	0	0	0.0	1.0	1.0	10.0	24.5	72.5	76.5
9.0	0	0	0.0	0.0	1.5	10.5	39.0	70.0	91.5
10.0	0	0	0.0	0.0	1.0	12.5	40.0	68.0	86.5

applied compounds by the plant leaf. Interpretations of the pattern of movement of monuron* and simazine* in potato slices (fig. VI, 5) and of the uptake and movement of 2,4-D* (fig. VII, 1), monuron* (fig. IX, 3, 4), and simazine* (fig. VIII, 1) by plant roots have also been illuminating. A continuing problem is the ready entrance of monuron* and simazine* into and through roots, presumably across the symplast of the endodermis - in contrast to their consistent failure to enter the symplast of leaves; applied to stems of bean and soybean seedlings they apparently bypass the phloem, enter the xylem, and move upward in the transpiration stream (fig. XI, 1). Histoautoradiographs using tritiated monuron indicate that this tracer is present in cell walls of the leaf but absent from living cells. However, the substituted urea and symmetrical triazine herbicides are powerful inhibitors of oxygen evolution in photosynthesis (Hill reaction), as shown by Cooke (1956), Wessels and van der Veen (1956), Moreland, *et al.* (1959), Todd (1960), Jagendorf (1958), Exer (1958), Gysin and Knusli (1958, 1960), Ashton, Zweig and Mason (1960), Ashton, Uribe and Zweig (1961), Roth (1958), Sweetzer and Todd (1961), and Sweetzer *et al.* (1961). Thus they must enter the chlorenchyma cells of leaves and migrate to the chloroplasts where photosynthesis takes place. That this occurs at amazingly low concentrations is indicated by the fact that these compounds are effective at dosages of from 1 to 4 pounds per acre, corresponding to concentrations of around 1 ppm in the soil solution. However, the dense labeling of foliage of plants treated with monuron* and simazine* by application to roots indicates a concentrating effect in the leaves resulting from the continued supply through the transpiration stream, the failure of retransport via phloem, and the loss of water from the leaves by transpiration (fig. VIII, 1; IX, 3, 4).

With regard to a possible aqueous route for entry of solutes from the cuti-

TABLE XI, 2
Weight in Grams of Growth of Bermudagrass Shoots
Sprayed January 11, 1951, with 0.5 per cent MH
Adjusted to a Series of pH Values

	Weight in grams †						
	Harvest dates						
pH	7/3/51	9/25/51	12/3/51	3/7/52	7/23/52	9/2/52	Average weight
(Untreated control)	51.5	81.9	12.5	14.0	24.2	10.0	32.3
2.0	16.7	59.0	15.2	18.2	22.2	9.5	23.9
3.0	17.1	58.0	15.4	18.5	19.2	8.0	22.7
4.0	18.5	40.2	16.6	25.3	22.0	8.6	21.9
5.0	19.5	18.7	11.7	21.3	28.3	8.6	18.0
6.0	17.1	7.7	7.9	9.3	10.9	3.9	9.5
7.0	13.9	7.0	5.9	0.0	0.0	0.0	4.5
8.0	15.8	0.6	7.9	14.0	15.5	4.1	9.7
9.0	20.2	25.2	19.3	24.7	27.9	11.6	21.5
10.0	18.5	37.1	11.6	13.7	31.8	10.6	20.6

† Values are averages of duplicate cultures.

cle into the interior, it is plausible to assume that the water in the leaf constitutes a continuum. This water-phase may reach the surface under saturated conditions, and hence application of aqueous sprays may present solutes for continuous diffusion into the leaf. That such a condition obtains in plants in saturated atmosphere is suggested by figure XI, 2, which shows four cotton seedlings treated with urea-C^{14}on one cotyledon. The two plants on the left were treated and put in a plastic bag to obtain a saturated atmosphere; the right-hand pair stood in the open greenhouse. The right-hand plant of each pair was steam-ringed below the cotyledons. In the greenhouse, only symplastic movement took place; in the plastic bags in saturated atmosphere water condensed on the treated spots and moved apoplastically to opposite cotyledons and into mature primary leaves. Symplastic movement was enhanced by the high humidity conditions (Clor, Crafts and Yamaguchi, 1962).

Figure XI, 3, shows coleus plants treated with 2,4-D* and amitrole* and placed in polyethylene bags to produce a saturated condition. Although differences in mobility of the two tracers are shown, the humid atmosphere has promoted apoplastic movement of amitrole* from the treated portions of leaves to untreated portions, to roots, and to growing axillary shoots. These two herbicides are much more effective in humid regions than in semi-arid regions.

Figure XI, 4, shows *Tropaeolum majus* plants treated with 2,4-D* (left) and amitrole* (right) in the greenhouse. Here the 2,4-D* shows typical symplastic movement whereas amitrole* has leaked from phloem to xylem so that leaves below the treated leaf show light labeling by apoplastic movement.

Fig. XI, 1. Apoplastic movement of monuron* in bean. A droplet of monuron* solution (20 µl containing 0.1 µmole was applied to the midrib just above the insertion of the petiole (left). The same amount was applied to the petiole, near the node (right).

When the leaf is under tension due to low atmospheric humidity, menisci must be drawn back for varying distances in the cuticular pores and hence air blocks will prevent union of an applied spray liquid with the water continuum and will inhibit entry of contained solutes. Crafts and Foy (1962) have proposed that a surfactant compatible with the cuticular lipoids may bring about a wetting of pore walls, a surrounding of the air bubbles, and hence a solubilization or displacement of the blocks. Effectiveness of a spray formulation in surmounting such a block must depend upon the amount of tension, and the ability of the surfactant to wet the specific cuticle.

Once union has been made between an applied spray droplet and the water continuum of the leaf, drying of the droplet must slow down or come to a halt and diffusion of the applied solute must continue for a considerable period of time. For a lipoid-soluble solute, diffusion across the cuticle and partition into the symplast should continue as long as the molecules remain unbound in the leaf surface.

Fig. X, 2. Effects of a saturated atmosphere and steam-ringing on distribution of sucrose* from urea* in cotton plants. Plants in photograph on left were enclosed in a polyethylene bag to give a saturated atmosphere; plants in photograph on right were in the open greenhouse. The right-hand plant of each pair was steam-ringed below the cotyledons before treatment.

The prolonged migration of solutes into leaves is shown by many autoradiographic studies. Figure IX, 1, shows barley treated with MH* and given 3-, 9-, and 27-hour treatment times. Time-series studies show the increased uptake and distribution of IAA*, amitrole*, and MH* that takes place during 1-, 4-, and 16-day treatment times in barley and bean. Jackson (1962) found this same effect in treating oxalis plants with phenoxy herbicides.

In view of these considerations it seems obvious that the plant body consists of two solid systems, the apoplast continuum and the symplast continuum, and that each of these systems plays an important role in the physiology of the plant. The symplast makes up the living stuff of the plant and hence is of critical importance in all plant functioning. The concept of the symplast as an interconnected continuum is all-important with respect to translocation. Since foods are synthesized in certain highly specialized cells and transported from these to all other living tissues, the distribution function of the symplast is of utmost significance. Many tracer molecules are mobile in the symplast, while others are subject to forces of retention within living cells. The mobility factors of a molecule determine whether it will have local or systemic distribution within the plant body. Thus, these factors must be understood to properly evaluate pesticide properties of new chemicals - and the autoradiographic method is invaluable in such studies.

The apoplast in turn is of critical importance, both in the physiology of plants and in the uptake and distribution of exogenous chemicals. All such chemi-

Fig. XI, 3. Coleus plants treated with 2,4-D* (left) and amitrole* (right). The left-hand leaf of each plant was treated on the lower surface (stomata present); the right-hand leaf on the upper surface (stomata absent). After treatment, plants were enclosed in polyethylene bags to develop high humidity. Treatment time was 4 days and dosage was 0.5 μmole per leaf; Tween 20, 0.1 per cent.

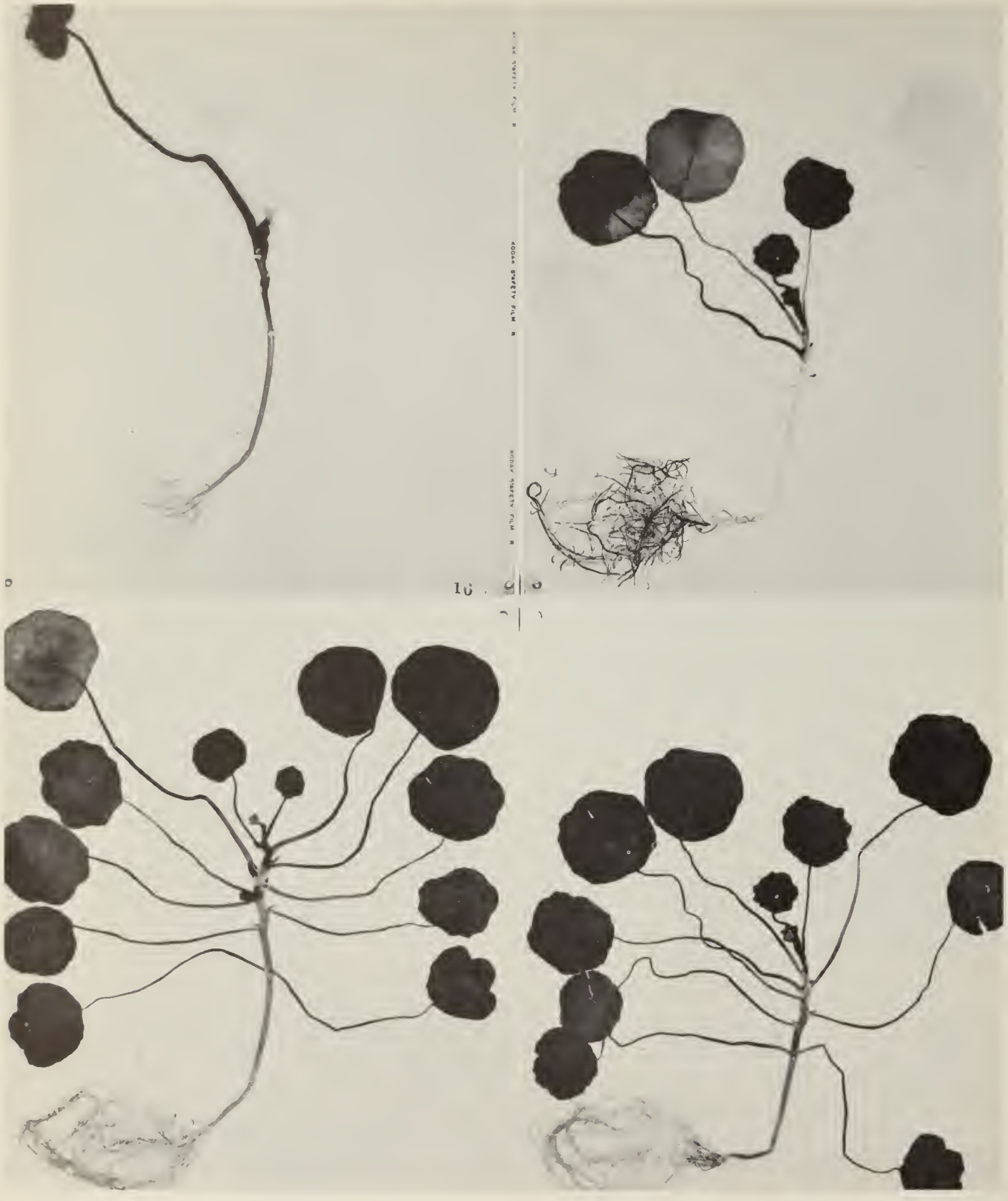


Fig. XI, 4. Nasturtium plants treated with 2,4-D* (left) and amitrole* (right). Dosage was 0.5 μmole per plant, time was 4 days; Tween 20, 0.1 per cent. The 2,4-D*-treated plant shows symplastic movement only; the amitrole*-treated plant shows symplastic movement plus some apoplastic, as a result of leakage from phloem to xylem.

cals enter the plant via the apoplast; water, salts and many pesticides undergo their major distribution via the apoplast; the apoplast makes up a large portion of the body of the living plant; a killed plant approaches the apoplast in properties; most pesticide residues reside on or in the apoplast. From the standpoint of the physiology of distribution, and from the standpoint of pesticidal action and pesticide residues, the symplast-apoplast concept is illuminating and useful - in fact, it may be a more practical concept than the cell concept in these particular fields.

Chapter XII. Studies on Herbicide Movement in Woody Plants

When 2,4-D was first used for control of deep-rooted perennial weeds it became evident that a new tool was available for controlling woody plants on range and forest areas. Trials along the eastern seaboard proved that 2,4-D would penetrate the foliage of woody plants and would, in some cases, translocate to the crowns, causing death of the meristematic regions and hence resulting in control. However, when the same type of applications were made in semi-arid regions, treatments were not as effective. Continued trials soon proved the efficacy of the basal-spray method, in which an ester formulation of 2,4-D in diesel oil was applied around the base of the tree in sufficient volume to run down and cover the crown where resprouting occurs.

Trials also proved the usefulness of the injection or cut-surface method in which the concentrated amine formulation is poured into cuts through the bark near the ground level. Many thousands of acres of weed trees have been killed in forest and range lands in the United States by this method. Airplane spraying of weed trees in cut-over forest to control broad-leaved species, and hence to release the young conifers in the stand, has also proved successful and is being used annually on thousands of acres in the U.S.A. - particularly against alders and willows, two species very susceptible to 2,4-D. A more recent method is to spray trees in the bud-swelling stage with ester formulations of 2,4,5-T; this has proved effective against maples and oaks, which are resistant to the toxic effects of 2,4-D.

Only one of these methods - the spraying of willows, alders and similar susceptible species in early summer - produces evidence for any extensive translocation of the herbicide; in the other three methods the toxic formulations are applied onto or very near the meristematic tissue responsible for resprouting. In the injection method, the chemical is placed in the sapwood and is carried principally into the tops via the transpiration stream; there is little evidence of phloem transport in these treatments.

In attempting to control woody species by means of translocated sprays a series of problems arise:

1. In treating evergreen species, application must be made at a time when assimilates are moving into roots.
2. In the case of deciduous species, the leaves must have passed the compensation point (exporting food materials), and they must be mature enough to resist contact action of the herbicide.
3. To control deciduous species, treatment must be made before soil moisture is exhausted and root-growth stopped.

Thus, these requirements set limits on time of application , concentration of the formulation, polarity of the molecules applied, and on the plant itself (with respect to soil moisture, temperature and growing conditions). The rules laid down from tests on deep-rooted herbaceous perennials must often be broken, and in the case of mixed-brush stands susceptible species may be controlled while resistant ones survive. Experimental work on woody plant species was undertaken with the above conditions in mind.

AUTORADIOGRAPHY OF WOODY PLANTS

Some of the methods used in studies on woody plants with labeled tracers are

illustrated in figures XII, 1, 2. These studies, using five toxicants and urea*, have produced much information about absorption and translocation of herbicides in such plants (Leonard and Crafts, 1956). Figures XII, 1, 2, show plant materials and autographs from the studies, and figure XII, 3, illustrates the method used in presenting information in graphic form. These studies on seven common woody plants of western brush-lands emphasized a number of points that were critical with respect to the successful use of 2,4-D on woody species in California. The following were the principal findings.

COYOTE BRUSH *(Baccharis pilularis)*, an evergreen shrub flowering in autumn. In February, 2,4-D* was absorbed but moved very little; translocation increased in April, diminished in May and June, and ceased in July. February and March treatments resulted in movement downward from treated leaves toward the roots. Tracer applied in April and later moved in both directions, with much more tracer located in the upper foliage.

ARROYO WILLOW *(Salix lasiolepis)*, a deciduous tree. Little translocation of 2,4-D* took place in this species before April 15, but from late April until late summer translocation was continuous. Translocation both upward and downward from treated leaves continued from late April until late September. Upward transport was predominant in April; by October all movement was downward.

WEDGE-LEAF CEANOTHUS *(Ceanothus cuneatus)*, an evergreen shrub flowering in spring. There was ready absorption of 2,4-D* by leaves but little translocation. Leaves, which were treated on their surfaces, were rapidly killed and this may explain the failure of tracer to move.

MANZANITA *(Arctostaphylos manzanita)*, an evergreen shrub flowering in midwinter (fig. XII, 2). Translocation, starting in March, was mostly downward. Bark of this species could not be peeled after June.

TOYON *(Photinia arbutifolia)*, an evergreen

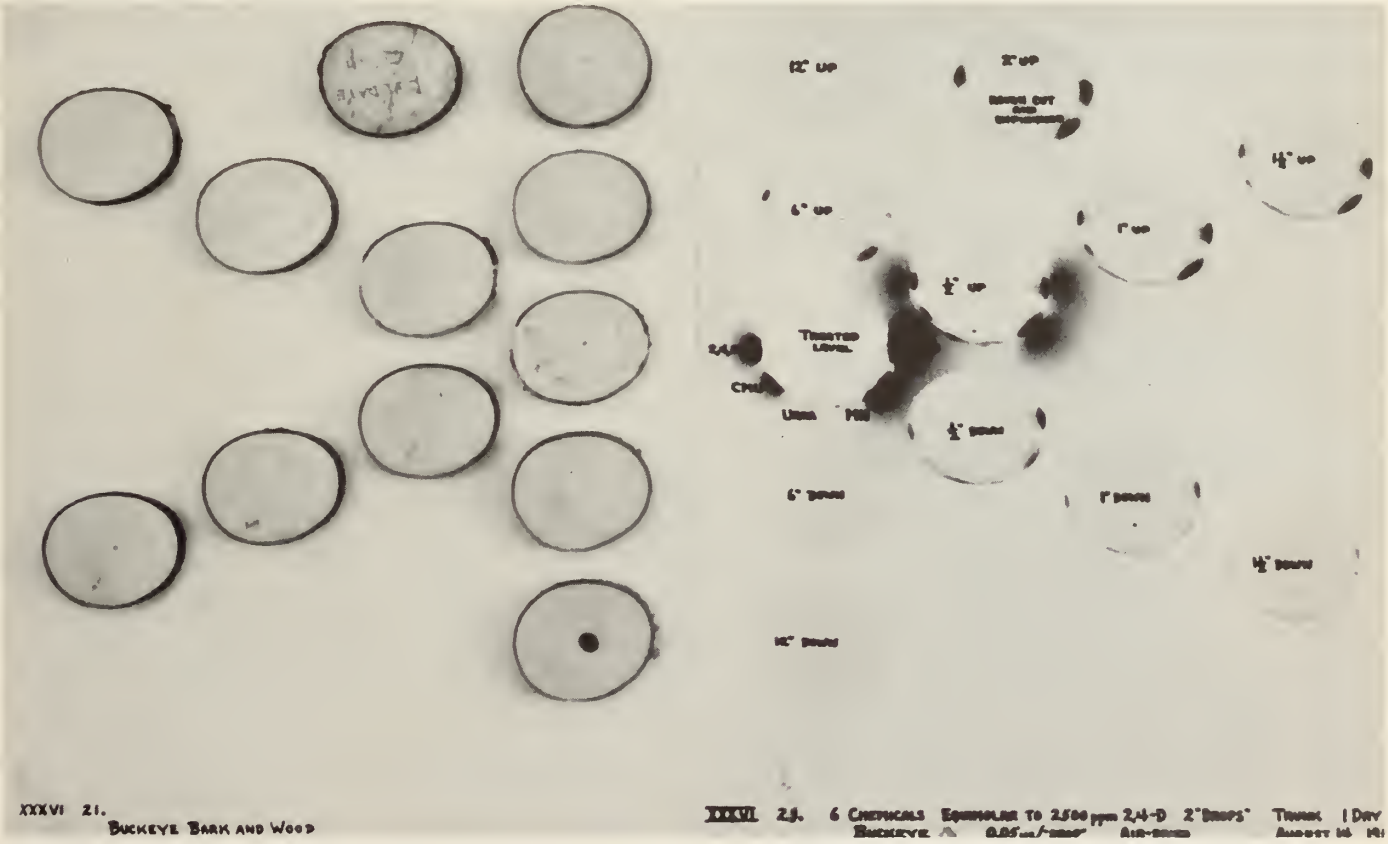


Fig. XII, 1. Cross-sections of buckeye stem (left) mounted and ready to autoradiograph; (right) autoradiograph of the mount. Film was reversed in photographing. The treatments appear as black spots; counterclockwise from left to right they are: 2,4,5-T*, monuron*, urea*, MH*, amitrole*, and 2,4-D*. At a level of 6 inches above the treatment, 2,4,5-T*, amitrole* and 2,4-D* can be seen; at 1-1/2 inches below 2,4,5-T* and 2,4-D* are present.

shrub or tree blossoming in early summer. Absorption and translocation of 2,4-D* was active from February through October. Transport was predominantly downward during February and March, upward in mid-spring and summer, and downward again in the fall. During late summer and early fall the autographs were light, indicating poor penetration of tracer.

BLUE OAK *(Quercus douglasii)*, a deciduous tree blossoming in early spring. Tracer studies were mostly negative. From March to June only five out of forty-two samples showed any movement, and the average distance moved was only 5.2 inches. Blue oak leaves are very sensitive to contact action by 2,4-D; this may explain the poor results in the experiments and the gener-

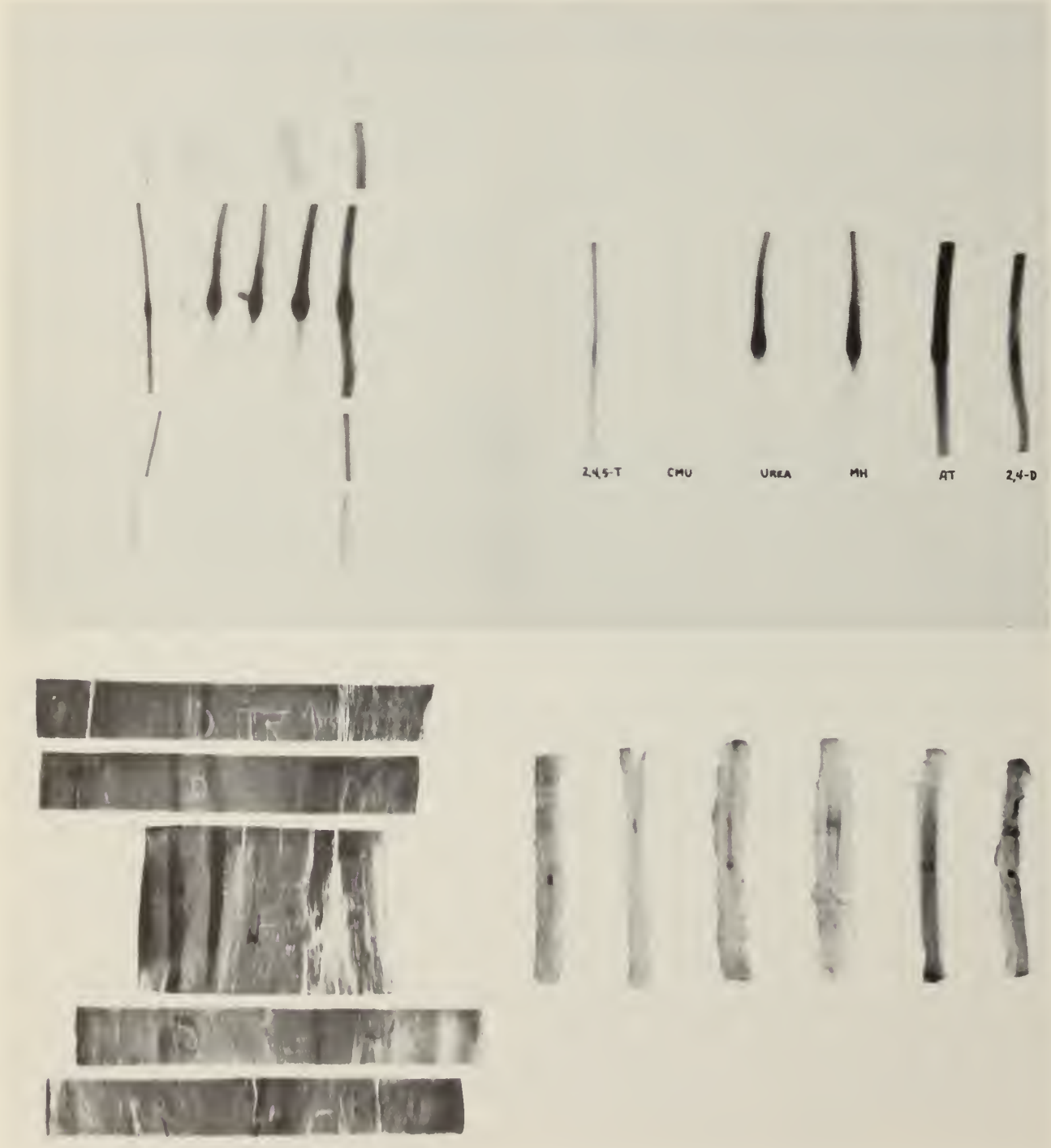


Fig. XII, 2. Autoradiographs (above) and mounted bark and wood (below) of manzanita treated with six tracers. From left to right, the chemicals as shown in the bark and wood autographs are: 2,4,5-T*, monuron*, urea*, maleic hydrazide*, amitrole* and 2,4-D*.

ally poor results of field application of this herbicide to blue oak.

LIVE OAK *(Quercus wislizenii)*, an evergreen tree blossoming in spring. Translocation was active from February through September. Movement was entirely downward in February, partially upward after new growth started in March, and largely downward during summer and fall.

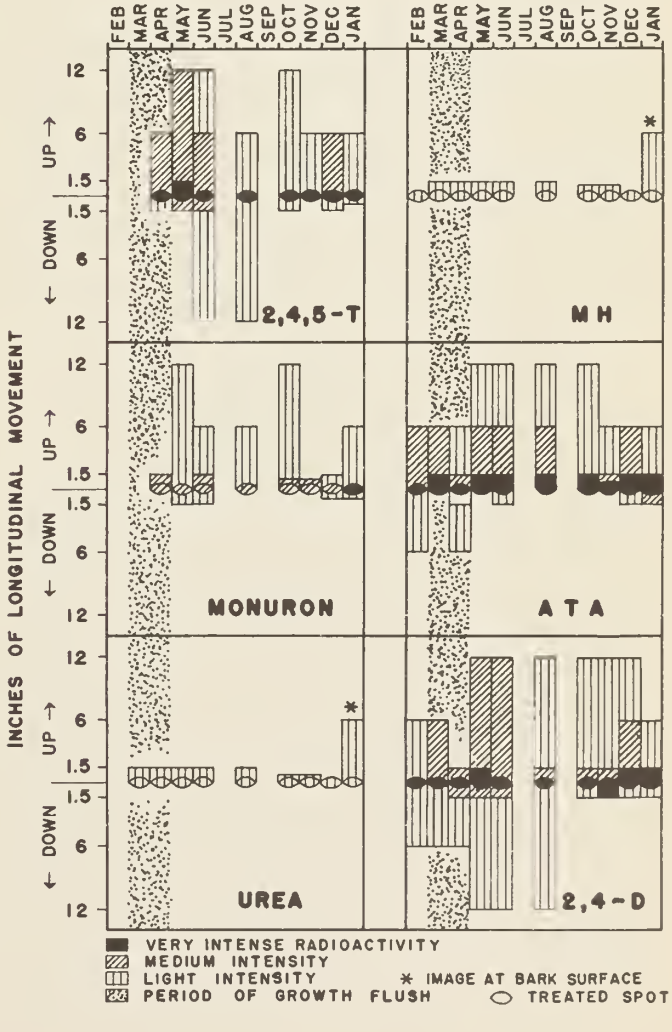
These studies, carried on in the field with native plants, emphasize some important points. Because early trials indicated that little 2,4-D* was penetrating the cuticle on the upper surfaces of leaves, later treatments were placed on the lower surfaces. Careful studies of the original autographs and of many later ones showed, however, that tracer will penetrate the cuticle in the absence of stomata. Penetration studies showed that the intact cuticle may protect the leaf tissues from a too-rapid absorption which might cause rapid injury of leaves and inhibition of transport. We suspect that this might explain the excellent results obtained with airplane application of an oil formulation of 2,4-D ester on wedgeleaf ceanothus, whereas our tracer trials placing the 2,4-D* on the lower surfaces of leaves resulted in little movement, and field application of 2,4-D ester in oil to blue oak usually fails because of excessive contact toxicity. This conclusion reemphasizes the significance of penetration and the relation of contact injury to uptake and transport. Many failures in the use of herbicides on woody plants may result not from lack of absorption but from too rapid an uptake, with resultant injury and breakdown of tissues providing the source of energy for translocation.

The relation of 2,4-D transport to food movement in plants is evident throughout the woody plant work, and the fact that only growing cells and tissues respond to 2,4-D is also important. It is evident that a thorough knowledge of the physiology of plants is necessary if one is to treat woody species with success.

Translocation in deciduous species is going on during much of the year, and probably year-round in evergreen plants; as long as plants are synthesizing foods and using them in growth, flowering, fruiting, and storage, translocation processes are going on. Failure to kill active plants having green foliage seldom results from failure of translocation; rather, it may result from lack of absorption, from translocation to the wrong tissues (flowers, fruits, or vegetative shoots), or from the inability of root cells to respond to 2,4-D. Because root response to 2,4-D involves active root growth, *available soil moisture is essential to successful treatment,* and this is true for all hormone herbicides.

The period for successful treatment of deciduous woody species may be relatively brief, occurring in spring or early

Fig. XII, 3. Translocation of six C^{14}-labeled tracers in the trunks of toyon through an annual cycle. Readings were made on rings of bark removed 24 hours after treatment and then dried and autographed. (Methods used are described in Yamaguchi and Crafts, 1959.)



summer when leaves have fully expanded and are mature enough to absorb actively yet are not too heavily cutinized. Weather conditions should be favorable, with moderate temperatures and adequate humidity. In seasons of low rainfall such periods may last a few weeks or only a few days; in extremely dry periods there may be no such time and chemicals will be ineffective, as was shown by the failure of chemical treatment of mesquite in Texas and parts of Arizona during a succession of dry years.

Continued spray programs on many species have proved that, in contrast to blue oak, arroyo willow - which thrives only in moist habitats - may be treated successfully from the time leaves expand in spring until late autumn. Several evergreen species may be treated in late winter or early spring while the previous season's leaves are still green and active, and at a time when soil moisture is amply available. Toyon and wedgeleaf ceanothus are two such species

With the introduction of amitrole, translocation studies soon proved this herbicide to be highly mobile in plants. Because its mode of action does not necessarily involve growing tissues it may be used as a translocated chemical during summer, when soil moisture may be relatively unavailable. Under conditions common in the American West, this considerably extends the period when woody plants may be controlled.

COMPARATIVE STUDIES ON TRACER UPTAKE AND TRANSPORT IN WOODY PLANTS

With the demonstration that different labeled tracers show different and characteristic behaviors in plants (pages 71 to 81), it became apparent that an extension of these studies of woody plants should open new paths to control of these weeds (Yamaguchi and Crafts, 1959). Accordingly, studies were set up to examine comparatively the uptake and distribution of 2,4-D*, 2,4,5-T*, amitrole*, maleic hydrazide*, monuron*, and urea*. Table XII, 1, shows the molecular weights and concentration of treatment solutions of chemicals used in these comparative studies.

Table XII, 1
Molecular Weights and Concentrations
Of Chemicals Used in Treating Woody Plants

Chemical	MW	Concentration, ppm
2,4-D	221.0	2,500
2,4,5-T	255.5	2,890
Amitrole	84.5	956
MH	112.0	1,267
Urea	60.0	679
Monuron	198.0	2,240

Three tree species were used - manzanita, toyon, and buckeye (*Aesculus californica*); the first two are evergreens, while buckeye is a deciduous species blossoming in early summer. Applications were to the active phloem of the trunk after removal of the outer bark (fig. II, 9). The autographed materials consisted of rings of bark and strips of wood taken in the region of treatment (fig. XII, 2), sections of stem (fig. XII, 1), and shoots. Experiments were carried on for 1 year. Figure XII, 3, shows a graph of the combined results of the whole test on toyon.

In this study, 2,4-D* and 2,4,5-T* moved downward in March, April, and May in buckeye; in March, April, May and June in manzanita, and June through August in toyon. In March and April the movement was mostly or entirely downward in manzanita and buckeye. From May until midsummer the downward movement gradually lowered and upward movement became prominent, especially in manzanita and buckeye. In late summer, fall and winter, movement in these two species came to a standstill, probably because of water stress. Seasonal trends were similar in toyon, except that some upward movement occurred throughout the year. Toyon, unlike manzanita and buckeye, grew at the bottom of a canyon where water was available and root growth could have continued throughout the summer months and into the fall.

Amitrole* movement downward was generally less than that of the phenoxyacetic acids, and was somewhat inconsistent.

Movement was most extensive in manzanita and continued from January to July; downward movement during these months was equal to, or less than, upward movement - from August through December movement was predominantly upward.

In toyon, downward movement of amitrole* was less than that of the phenoxy compounds and occurred mostly from February to April. In buckeye, movement was also restricted and took place mainly in June. Amitrole* had a strong tendency to fix in the xylem tissues and movement was only in the upward direction; this was true of all three species.

Maleic hydrazide* and urea* had similar distribution patterns. They moved downward 12 inches or more in manzanita during January and February and again in May; upward movement was even more erratic. MH* and urea* are both highly water soluble; the treated spots in many cases were low in radiation intensity; probably these compounds diffused readily along the apoplast and were flushed up the xylem in low concentration in the transpiration stream. This indicates, in contrast to 2,4-D*, 2,4,5-T* and amitrole*, a very low tissue-retention and relatively free mobility. In toyon and buckeye, MH* and urea* were almost completely carried away.

In the January treatment of toyon, light images of radiation from MH* and urea* were found at the outer surface of the bark up to 6 inches or more above the treated spots. Transpiration was low at this time, and apparently tracers were carried laterally from the vascular channels across phloem and cortex and deposited at the surface where water loss by evaporation was occurring. Later, when transpiration increased, tracers were probably dissipated over so much area that they could not be detected by the autoradiographic method. Evidently, free mobility of a tracer in such tissues is an indication of lack of retention by living cells.

Monuron* showed essentially no downward movement in these tree trunks. Upward movement was moderate in toyon, less in buckeye, and almost lacking in manzanita; even the treated spots were faint. This compound apparently moved with great freedom in the apoplast and was largely carried away to the tops of the trees in the transpiration stream; it did not enter the phloem and move in a downward direction.

Following the studies on tracer movement in tree trunks, an experiment was set up using toyon and manzanita seedlings (Yamaguchi and Crafts, 1959). Here, chemicals were confined to single small plants so that the distribution of each could be determined. Applications were made to stems in two ways: by making a small cup of aluminum foil around the stem and filling it with treatment solution, and by cutting away the outer bark (as had been done in the previous study) and treating by means of an aluminum foil cup which surrounded the bared area (fig. II, 9, right). The cups were sealed in place with lanolin and 60 microliters of treatment solution were used in each treatment.

Autographs of these plants show the distribution obtained by this method (fig. XII, 4, 5). The autographs also showed one consistent difference between the treatment on old tree trunks and on young seedlings: a predominance of upward movement in the young plants. The young trees were growing rapidly and they might be compared with the older trees during their spring flush of growth. In both cases 2,4-D* showed movement both upward and downward; in the seedlings, downward movement was much less prominent. Removal of outer bark considerably enhanced absorption of the tracers in toyon.

LATERAL MOVEMENT OF TRACERS IN WILLOW

Knowledge of the relative mobility of different compounds both longitudinally and laterally in woody stems is needed in order to interpret the distribution of pesticides in plants treated via the foliage and via the roots. To obtain information on these processes, experiments were performed with willow stems of 3/4-inch diameters. The autographs of the phloem and xylem tissues immediately above and below treated spots show that monuron*, MH*, and amitrole* move principally upward. Although such movement could have occurred only in the xylem, evidence for presence of the tracers is present in both tissues. In the case of 2,4-D* there is evidence for both upward movement in the xylem and downward move-

Fig. XII, 4. Toyon shoots treated on the stem with 2,4-D* Shoot on the left was treated by applying tracer solution through a cut in the bark; shoot on right was treated by application of tracer to the intact bark.

Fig. XII, 5. Toyon shoots treated with monuron*. The left-hand shoot was intact, the right-hand shoot was treated through the cut bark. In contrast to leaves in fig. XII, 4, the very young leaves here are lighter than the old - an indication of apoplastic movement of monuron*.

ment in phloem, and both tissues contained the tracer. This confirms the evidence presented by Stout and Hoagland (1939) for rapid lateral movement of tracers across the cambium. When cuts were made across the bark at positions 1 inch apart and treatment was applied between the cuts, all four compounds moved upward; none moved downward. This proves that the tracers penetrated into the xylem and moved in the transpiration stream; because the phloem was blocked by the cut below the treatment no downward movement of 2,4-D* occurred.

Autographs were made of stems from which the bark was pried loose over a distance of 4 inches and then pressed back and bound in place with masking tape. The autographs, when compared with those of treatments on stems with intact bark, showed that there is almost no difference between movement of monuron* and MH* through stems in which the bark was separated and through intact stems. In the case of amitrole*, there was labeling in the phloem and xylem and upward movement in xylem only. The results with 2,4-D* are most illuminating. Here, movement took place in the phloem in both directions but was strongest in the downward direction. In the xylem there was little upward movement; below the treated spot there was little evidence of tracer in the region where the bark was lifted, and there was a strong labeling below this region. Evidently the downward movement took place in the phloem, but in the regions of intact bark lateral movement to the xylem was very rapid. Separation of the bark (phloem) from the xylem evidently injured the symplast so that even though the tissues were bound together again lateral movement was inhibited. This is a complementary picture to the one obtained by Stout and Hoagland (1939) for movement via the xylem.

These studies show that movement may take place in three distinct ways: (1) upward in the transpiration stream (aqueous continuity in the apoplast), (2) downward by way of the phloem (specialized channels of the symplast), and (3) laterally via the cell walls (apoplast) from phloem to xylem and *vice versa*, and into living parenchyma (accumulation). The more polar compounds bypass the symplast and move via the apoplast and the specialized xylem conduits; the more lipophilic ones are absorbed and moved in the symplast. These studies show clearly the possible relations between the three above modes of transport. It is well to keep in mind that all applications, practical and experimental, are to the apoplast.

Much knowledge of transport phenomena in woody plants has been acquired, and already some of it has been applied to field practice. Meanwhile there is much room for more studies and for amplification of our knowledge of tree physiology through autoradiography. Already, hundreds and thousands of acres are sprayed in various tree-farming and range-management operations, and in the future millions of acres will be sprayed annually. Thanks to such practices, utilization of our forest and range lands will be greatly improved.

Chapter XIII. Translocation in Grasses and Coffee Plants

TRANSLOCATION STUDIES ON GRASS SPECIES

Because some grass species are highly important crops, and because some are obnoxious weeds, studies on certain of them were initiated early in our program. Our earlier studies were designed to elucidate the mode of action of certain herbicides. For example it has been shown that 2,4-D* moved about as readily in barley as in bean and hence that the selectivity of 2,4-D was not directly related to the uptake and transport of this compound (figs. VI, 2, 3; VIII, 1, 2). These studies proved, furthermore, that amitrole* moves more readily than 2,4-D* in barley, and that MH* moves very freely and actually migrates from phloem to xylem and hence may circulate. Monuron*, on the other hand, does not enter and move in the phloem (fig. VI, 3). Barley was used in these experiments as an example of a monocotyledonous species - one that has scattered vascular bundles and a unique nodal anatomy as contrasted with dicotyledonous species.

Peterson (1958) applied droplets of 2,4-D* solution to all of the main leaves of barley plants in various stages of growth; a consistent change in the pattern of distribution occurred at about the four- to five-leaf stage, and far less 2,4-D* reached the root system than in treatment at earlier stages. Van der Zweep (1961), studying 2,4-D* movement in barley, also found restricted movement to roots after the three- to four-leaf stage. This restriction was not related to the change from vegetative to reproductive growth of the growing point; it was more probably the result of a shift in the activity of various sinks, those in the roots being superseded by the growing leaves and inflorescences.

A more recent study on movement of C^{14}-labeled assimilates in wheat is that of Quinlan and Sagar (1962). In the early stages of development of the wheat plant assimilate from leaves on the main shoot was transported throughout the plant but accumulation occurred in meristematic regions. Assimilates produced by leaves of newly-formed tillers were distributed mainly to the meristematic regions of those tillers, although sometimes there was movement into the rest of the plant. In the later stages of development of each tiller the labeled assimilate was restricted to the tiller itself and to any very young tillers attached to it. After emergence of the head, the distribution pattern from the youngest and second-youngest leaves changed: movement was predominantly toward the head from the flag leaf and toward the roots from the leaf below the flag leaf.

Quinlan and Sagar found little evidence for a block in the movement of assimilate from top to roots in the wheat plant. Crafts (1959*b*) found free movement of amitrole* from leaves to roots in barley, some restriction on the part of IAA*, and almost no movement of 2,4-D*; he concluded that the block was physiological, and that it was related to the avidity with which meristematic cells accumulated the latter two tracers.

Quinlan and Sagar also found evidence for movement of labeled compounds from young into older leaves. While concentrations were low, evidence for their presence is certainly definite (Quinlan and Sagar, 1962, plates 20a, b, d). They attempted to increase and decrease transpiration rate and to prove that this

is related to xylem movement. While their methods should have changed the rate of water loss, even surrounding the leaf with water will not prevent loss if the leaf is in the light. Similar labeling of old leaves in our experiments has been attributed to the movement of degradation products of assimilate metabolism in the transpiration stream. Instead of attempting to stop transpiration in order to solve this problem, xylem sap should be analyzed for C^{14}-labeled compounds - organic acids, for example.

The work described below was on the basic organic nutrition of different grass species as it is related to the growth of forage plants.[2] Compounds labeled with C^{14} were used and an attempt was made to discover how various plant parts fit into the economy of the plant as a whole. Earlier work with perennial ryegrass (*Lolium perenne*) had shown that the translocation of C^{14}, introduced into the leaf as $C^{14}O_2$ under photosynthetic conditions, was very rapid and that the isotope moved to all growth-centers of a plant in about an hour (Forde, 1959). A most striking feature of ryegrass is the absence of internodal elongation during the vegetative stage. In the present study it was desired to contrast translocation in ryegrass with that in Bermudagrass (*Cynodon dactylon*), which has internode elongation above ground by a nonstorage organ, quackgrass (*Agropyron repens*) which has internode elongation in a storage organ, the rhizome, and corn (*Zea mays*) which has vertical internodal elongation. (After initial investigation the last species was omitted because when at all mature it was inconvenient for this type of study.)

Under controlled conditions (75 degrees Fahrenheit and 70 per cent relative humidity) a 2 µl drop of radioactive solution was applied to the lamina of the leaf at a point between 1/2 and 1 centimeter above the ligule (fig. II, 11). The compounds used were urea*, amitrole* and MH*. Carbon-labeled ammonium thiocyanate was used in one series but unless its name is mentioned, it can be assumed that only the first three compounds were used. Clor (1959) showed that urea* is rapidly split by the urease present in cotton to give ammonia and, under photosynthetic conditions, the products of normal $C^{14}O_2$ fixation by photosynthesis, the labeled compound translocated being sucrose. Urea* has a number of advantages over $C^{14}O_2$ in application, the principal one being that urea* can readily be applied as an aqueous solution. The translocation pattern of the products of urea* breakdown is typical of phloem movement, with little xylem movement. In earlier studies amitrole* had been found to move with the assimilate stream in the phloem, movement being typically from the point of synthesis of photosynthate (the source) to the point of utilization (the sink). Maleic hydrazide* follows this pattern to a considerable extent, but also has been shown to move in xylem to a marked degree after leaf application, presumably due to leakage from the phloem to the xylem.

Unless otherwise stated, plants in the translocation studies were grown in sand culture with periodic waterings with normal Hoagland's solution. Treatments lasted from 1 to 96 hours, after which time the plants were carefully harvested and killed with powdered dry ice. The frozen plant material was freeze-dried, mounted on paper, and an autoradiograph was then prepared.

After observing the translocation pattern in the whole intact plant, experiments were conducted in which parts of the plant were either blacked-out completely (using black paper screen) or defoliated. The radioactive solution was always applied to a nonscreened or nondefoliated leaf. The purpose of these experiments was to see whether other sinks could be created by interfering with the production of photosynthate, thus modifying the translocation pattern.

EXTRACTION OF THE PRODUCTS OF UREA* APPLICATION

Corn and ryegrass plants were treated with urea* using the methods described by Clor; after some hours (3 and 6 hours for corn; 3, 9, and 27 hours for ryegrass) plants were harvested, the treated spot

2. Work carried out by Bernard Forde and incorporated in a thesis for the partial satisfaction of requirements for the Ph.D. degree, University of California, Davis, California (Forde, 1963).

discarded, and the remainder of the plant divided into treated leaf, the rest of the shoot, and the roots. These fractions were homogenized in boiling 80 per cent ethanol and the supernatant, following concentration by evaporating under reduced pressure, was spotted on chromatography paper. Urea* and sucrose* were spotted singly on the same paper and also on top of a spot of extract. The chromatograms were run in butanol-acetic acid/water (4/1/1.5) dried, and autoradiographed. With corn, sucrose was the predominant labeled compound extracted (as evidenced from a comparison of the Rf of the known sucrose and the extract spot), and urea* was not detected in any of the fractions isolated. With ryegrass it was found that some urea* could be detected in the extract from the treated leaf though it could not be detected in the remainder of the plant, and sucrose was the main compound identified by cochromatography.

Extracts from the treated ryegrass leaf were also run in two dimensions using first phenol/water (80/20) and, after drying, butanol/propionic acid/water (623/310/437). Following autoradiography the compounds detected in the 3- and 9-hour treatments were compared with the compounds detected from a red kidney bean plant treated with $C^{14}O_2$. Apart from the presence of urea* in the ryegrass extract, no marked qualitative or quantitative difference could be detected between the urea* fixation pattern and a $C^{14}O_2$ fixation pattern. Apparently it is safe to assume that the labeled products of urea* application translocated from the treated leaf, under photosynthetic conditions, are similar or identical to those from normal photosynthesis.

CORN EXPERIMENTS

Young plants with about four mature leaves were used and the solution of labeled compound (including Tween 20) was applied as a drop to the youngest mature leaf, or to the next leaf below it. No consistent difference was discernible in the amount of translocation from either leaf. The C^{14} derived from urea* moved into the roots and into the meristematic regions in 3 hours, though in fairly small amounts. This pattern of phloem movement to the active sinks was accentuated after 6 hours and even more so after 24 and 48 hours. Amitrole* showed a similar pattern of movement, but moved more slowly than the compounds derived from urea*.

The movement of MH* was somewhat irregular, but appeared to be slower than the sucrose* from urea*. There was also some evidence of xylem movement, as shown by the presence of the isotope in mature leaves.

PERENNIAL RYEGRASS EXPERIMENTS

Plants with about seven mature leaves on the main tiller were treated on the designated leaf with either labeled urea, amitrole, or MH for 3, 6, 12, 24 or 48 hours. The following leaves on different plants were treated at each of the times noted: the youngest mature leaf on the main tiller, the leaf two below the youngest mature leaf on the main tiller, the youngest mature leaf on a large side tiller and the youngest mature leaf on a small side tiller. In addition, one tiller was either shaded or defoliated 24 hours before an unshaded or undefoliated young mature leaf was treated. Movement was rapid, with the first two compounds being largely restricted to the phloem and the third compound moving in both the phloem and the xylem.

BERMUDAGRASS EXPERIMENTS

Plants raised from cuttings and with the main stolon about 2-1/2 feet long were treated for 3, 6, 12, 24 and 48 hours on one of a number of leaves. The plants were growing vigorously and had many side tillers. The following leaves were treated: the third mature leaf counting from the tip of the main stolon, the fifth mature leaf behind the main stolon tip, and a leaf inserted directly on the main stolon somewhere between at least two rooted nodes. Leaves were also treated on a large side tiller, on a small side tiller which was rooted below its point of attachment to the main stolon, and on a small side tiller not rooted below its point of attachment to the main stolon. Shading and defoliation experiments were made with all three chemicals for 12 and 48 hours following the pretreatment for 24 hours - that is, plant parts were shaded or defoliated 24 hours before treatment.

The most striking effect was the

strongly polarized movement of compounds towards the growing point of the main stolon. Except where a portion of the stolon itself was meristematic proximal to the treated leaf, there was virtually no movement of C^{14} back towards the main root system. This effect is illustrated in figure XIII, 1. Where a small tiller on the main stolon was treated, it translocated material strongly into the main stolon and some activity passed into all tillers progressing towards the stolon

Fig. XIII, 1. Autoradiographs (above) and mounted plants (below) of bermudagrass; treatment with urea*. Plant on left was treated for 12 hours on a mature leaf inserted on the main stolon between two rooted nodes. Center plant had similar treatment on the sixth mature leaf from the tip of a minor stolon. Plant on right was treated for 48 hours on a mature leaf on the main stolon between two rooted nodes.

tip. Shading or defoliation for 24 hours before treatment did not appear to affect this pattern but it was found possible to reverse the pattern by shading or defoliating for 4 days before treatment with the isotope, with shading continued during period of treatment. Defoliation consisted of cutting off the laminae of all mature leaves 4 days before treatment and removing any regrowth 2 days before treatment and on the day of treatment. Figure XIII, 2, shows translocation of sucrose

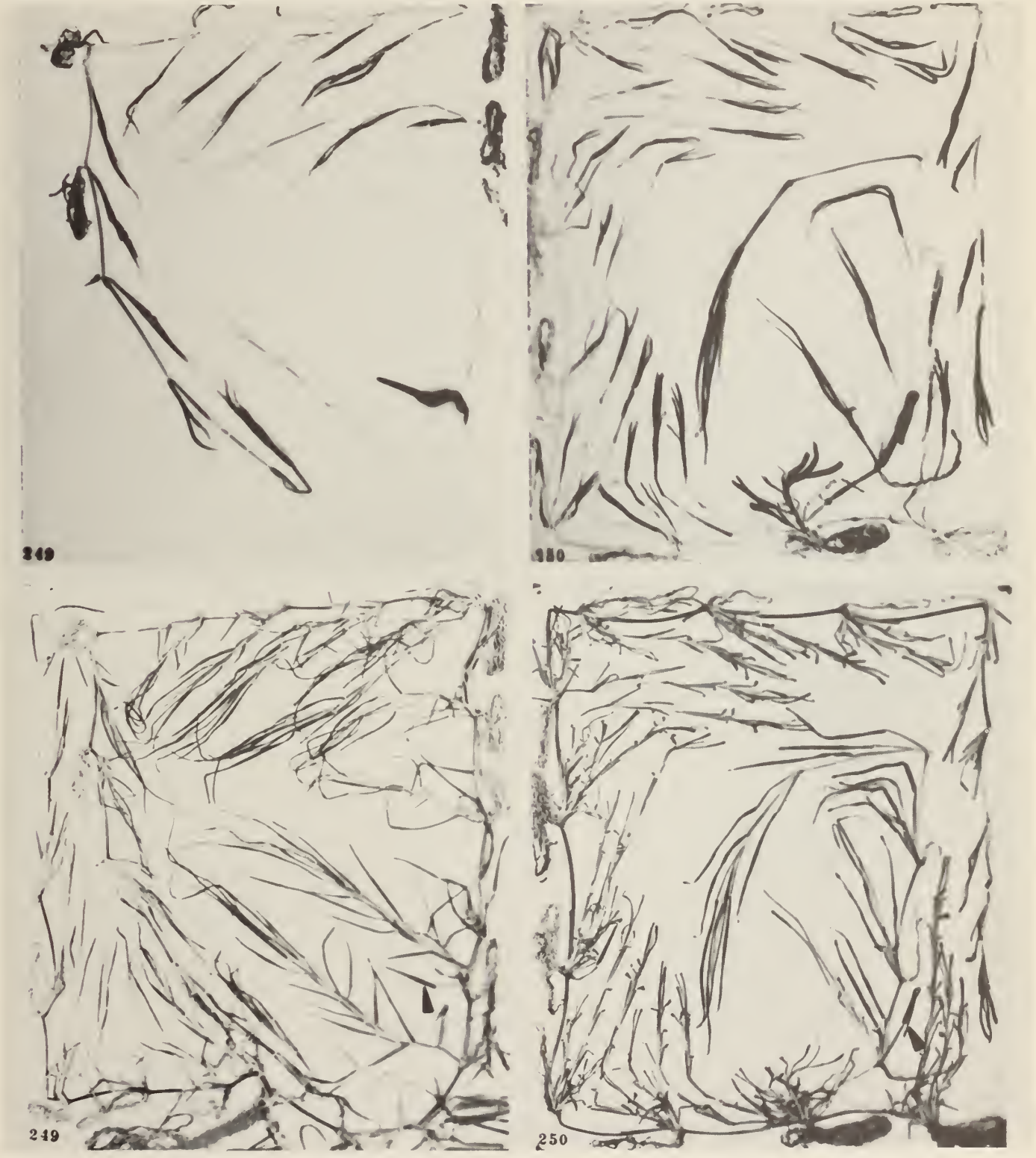


Fig. XIII, 2. Autoradiographs and mounted plants of bermudagrass; treatment, urea* for 48 hours. Plant on left had all but the treated stolon darkened for 4 days before and during treatment. Plant on right had all but the treated stolon defoliated 4 and 2 days before treatment, and again at time of treatment.

in a shaded and in a defoliated plant.

QUACKGRASS EXPERIMENT

Plants with well-developed rhizome systems were treated for either 12 or 48 hours with one of the three compounds. Leaves treated were as follows: the youngest mature leaf on the main tiller, the leaf two below the youngest mature leaf on the main tiller, the youngest mature leaf on a medium-sized side shoot. Shading and defoliation treatments were also carried out. Translocation occurred fairly freely within a treated tiller and from a main tiller through a rhizome to a small tiller. However, little or no movement occurred in the reverse direction. There was strong movement of the derivatives of urea*, and of amitrole* into the young leaves, the roots, and into the actively growing rhizomes. In addition to phloem movement, MH* showed considerable movement in the xylem. Shading or defoliation of a single shoot 24 hours before treatment did not appear to greatly alter the pattern of movement. Peterson (1962) has studied the movement of amitrole* and dalapon* in quackgrass.

TRANSLOCATION STUDIES ON THE INTERCALARY MERISTEM

In earlier studies it had been found that when $C^{14}O_2$ was applied to an immature leaf of perennial ryegrass, the isotope moved strongly to the region of the intercalary meristem but did not pass across it to the remainder of the plant unless the leaf was almost mature. In view of statements in the literature of this subject about intercalary meristems being a block to translocation, an attempt was made to study movement of photosynthate in this region. A blockage or constriction might be caused by a partial or entire discontinuity in the phloem, or by metabolic activity in the area using photosynthate before it has time to pass across the meristem, or by a combination of the two. To investigate anatomical constriction or blockage, serial transverse sections were made of paraffin-embedded ryegrass material. Sections were made of the youngest mature leaf, and of all younger leaves, from their insertion into the main stem to a point about 7 mm above the level of insertion. The sections were stained with Sharman's tannic acid/iron alum stain which distinctly shows the phloem. The numbers of active phloem elements in the midrib and the lateral bundles on one side of the leaf were counted; an active phloem element was arbitrarily defined as one in which the blue stain was visible around the entire periphery of the cell, and one in which the cell was not crushed. The results indicate a considerable constriction of the phloem in the region of the intercalary meristem, and marked decrease in the number of phloem elements as compared with the more distal sections. In one midrib the number of phloem elements decreased from about fourteen cells to three. Some bundles are represented in the meristem by procambium only, while higher up they contain protophloem, and yet smaller bundles are present higher up and absent in the meristematic region.

These studies indicate that the restriction of the phloem conduits in the region of the intercalary meristem may constitute a physical block. However, the dense labeling of this region with C^{14}-labeled sucrose (derived from urea*) - in contrast to less intense labeling in more mature regions (fig. VI, 3) - seems to indicate an intense fixation of sugar by the growth processes, and this would slow down the linear rate of translocation. It seems likely that restricted phloem and high meristematic activity may both serve to block translocation in this region.

TRANSLOCATION IN COFFEE PLANTS

Translocation of a number of tracers in coffee plants has been shown to follow certain specific patterns (Pereira, Crafts and Yamaguchi, 1963). Amitrole* is highly phloem-mobile, quite readily moved via the xylem, subject to redistribution in the phloem, and absorbed slowly by roots. Dalapon* is readily phloem mobile, is slowly absorbed by roots, is retained by root tips but not by mature roots, and shows only restricted apoplastic movement in leaves.

Maleic hydrazide* is readily translocated with foods in the phloem, is somewhat restricted in the xylem, and is absorbed and transported to the xylem slowly by roots. MH* tends to leak from phloem to xylem and is readily redistributed from old to young leaves via phloem. 2,4-D* is avidly accumulated and retained by living cells, hence its distribution

in normal plants may be limited; only under optimum conditions of leaf penetration and food movement may it be moved from foliage to roots. 2,4-D* is not readily moved from roots to foliage via the xylem. Monuron* is non-phloem-mobile, but readily xylem mobile. It displays strong apoplastic movement in leaves and moves readily through roots and into tops when applied to roots in the culture medium.

The C^{14} label of urea was readily moved via the phloem in coffee; it was transported from illuminated leaves, probably in the form of sucrose. In shaded leaves, labeled urea moved mainly in the apoplast. $Calcium^{45}$ was not translocated via the phloem; it was readily absorbed by roots and moved via the xylem. It is accumulated in leaves and immobilized, showing no redistribution. $Phosphorus^{32}$ did not penetrate coffee leaves readily, hence its distribution via phloem could not be determined with certainty. $Phosphorus^{32}$ was accumulated by roots and moved only slowly to tops. $Zinc^{65}$ was less mobile in coffee than in barley and bean; it was less actively absorbed and moved by roots than was monuron.

The translocation of pentavalent arsenic as arsenic acid-As^{77} was studied in coffee. Leaves at various positions were given 4-day treatments by droplet application. Autographs showed the movement of small quantities both acropetally and basipetally from mature leaves. Active sinks in buds and small growing leaves and in root tips accumulated the tracer; all mature leaves were bypassed. Treatment via the culture solution resulted in movement to all mature leaves; most of the As^{77} applied to roots was retained in these organs however. General distribution of As^{77} resembled that of P^{32} and amitrole*.

These studies with coffee reveal individual idiosyncrasies of the different tracers, but also show patterns remarkably similar to those established in barley, bean, grass species, and zebrina. The repeated observation of the relatively free phloem mobility of amitrole, the restricted movement of 2,4-D as related to metabolic accumulation, the free phloem mobility and slow leakage to xylem of MH and dalapon, the lack of symplastic movement of monuron and calcium - these and many other characteristic behavior patterns have been found in coffee. Gradually there is evolving a consistent picture of solute transport in plants indicating the existence of a source-to-sink pattern of distribution of foods and tracers via phloem in plants, a variable pattern of uptake rates by roots, a consistent view of root-transport mechanism related to solute species, and a redistribution system keyed to the supply of needed nutrients to all living cells. Pesticide molecules foreign to plants fall into the various patterns, and their distributions often provide keys to their local or systemic action.

Chapter XIV. Translocation of Some Amino Acids in Two Barley Varieties

In studies on the common barley variety Atlas and a mutant Atsel selected from a dalapon-treated population of Atlas, Wijewantha and Stebbins (1964) found that adding arginine to the culture of the mutant barley in mineral solution culture shifted the expression of the phenotype of the mutant to almost normal. Since this implies uptake and distribution of the amino acid from the culture medium into the growing organs, it seemed useful to initiate a study using C^{14}-labeled amino acids by the technique of autoradiography.

Studies have been made using tryptophan in a dosage series involving applications of 0.05 μmole, 0.2 μmole and 0.8 μmole of tryptophan-C^{14} having a specific activity of 6.64 mc per μmole to both Atlas and Atsel barleys. One set of plants in this 1-day series indicated insufficient uptake for satisfactory comparison; a second set which ran for 4 days proved satisfactory (fig. XIV, 1*a,b*). This preliminary trial showed that tryptophan was absorbed and translocated via both leaves and roots with about equal

Fig. XIV, 1a, 1b. Autographs and mounted plants showing distribution of tryptophan* in Atlas (left) and Atsel barley (right) treated via the leaves (1a) and via the roots (1b). Dosages in each treatment were, left to right, 0.05 μmole, 0.2 μmole and 0.8 μmole per plant; treatment time was 4 days.

TABLE XIV,1
Label Locations, Specific Activities and Exposure Times
Of Amino Acids Used in Translocation Studies
On Atlas and Atsel Barley

Acid	Label location†	Specific activity mc/mmole	Exposure, days
DL-arginine	quanido	2.2	6.4
Glycine	C-2	3.0	4.7
L-histidine	uniform	9.3	1.5
DL-lysine	C-1	8.95	1.5
DL-phenylalanine	C-3	1.3	11.0
DL-tryptophan	C-3	6.64	2.1
L-valine	C-1	5.73	2.5
DL-valine	C-1	6.05	2.3

† Experimental time periods were 1, 4, and 14 days.
Applications were made to leaves and roots. (Cf. chapter 8.)

facility; of the various chemicals tested so far, tryptophan most resembles amitrole and is quite different from the phenoxyacetic acids and the substituted ureas and symmetrical triazines.

In application by both methods the resulting images show a good correlation between dosage and amount of tracer absorbed and translocated. Comparing the images produced by the three dosages it seems that any dosage from 0.05 to 0.2 µmole would prove satisfactory. For the following comparative study with eight amino acids, a dosage of 0.1 µmole was chosen.

Table XIV, 1, lists the amino acids used and specifies the location of the labels and the specific activities. Treatments involved 0.1 µmole per plant, with exposure times on the films adjusted to give standard images.

COMPARATIVE MOVEMENT OF EIGHT AMINO ACIDS IN TWO BARLEY VARIETIES

ARGININE Uptake of this amino acid by barley leaves was strong, with rapid phloem movement and light apoplastic movement.

GLYCINE Uptake of glycine by barley leaves was strong, with rapid and continued phloem movement into growing leaves and roots. Evidence for continuing distribution in the 4-day plants was positive; in the 14-day plants labeling weakened in the young leaves, probably because of exhaustion of supply of mobile tracer. In the 14-day plant, concentration was medium in mature roots and high in young roots. Apoplastic movement in the treated leaf was medium in intensity.

Root application of glycine resulted in medium movement to tops in Atlas barley, and light movement in Atsel. There was evidence for thorough distribution in the 1- and 4-day treatments; apparently the supply to the roots became exhausted in 14 days.

L-HISTIDINE If adjustment of exposure time of the film to specific activity to give a constant image density (see table XIV, 1) is taken as a basis for comparison, histidine was the amino acid most strongly absorbed by leaves. Movement via phloem to developing leaves and roots was very strong; there were indications

of continued distribution in the 1-day and 4-day treatments and of lowering availability in the 14-day plants. The two barley varieties had similar responses.

Movement of histidine from roots to tops was of medium intensity, with evidence for complete distribution in the 1- and 4-day treatments. Evidently, roots of barley accumulate and hold a large part of the applied dosage within the first 4 days; there seemed to be little more tracer in the tops after 14 days.

DL-LYSINE Absorption and movement of lysine was second in intensity to histidine, with strong images of all leaves of both barley varieties. Continuous movement is evident in the 1- and 4-day trials; the tracer supply was evidently exhausted by the 14-day time period. Accumulation of translocated lysine in roots was high in the growing regions of the 1- and 4-day plants, and only medium in roots of the 14-day plants.

Movement of lysine from roots to tops gave only light labeling; accumulation in roots was high - evidently release from symplast to apoplast was not free in the case of this amino acid. There is evidence for secondary movement via phloem in the 1- and 4-day plants.

DL-PHENYLALANINE This amino acid resembles glycine in its distribution in barley; absorption and movement are strong; prolonged movement is evident but not prominent; apoplastic movement in the treated leaves varied from medium to strong; availability as shown by the young leaves of the 14-day plants was limited by this time.

Accumulation of phenylalanine by barley roots was strong throughout the 14-day period; transport to the tops was medium to light; redistribution was not prominent in the short treatment periods and was lacking in the 14-day plants. The veins of the leaves of the plants treated via roots were prominently labeled, indicating strong accumulation by phloem and border parenchyma cells.

DL-TRYPTOPHAN Phloem movement of tryptophan from treated leaves to tops and roots of barley was in the medium range; mature roots were lightly labeled; con-

Fig. XIV, 2a, 2b. Distribution of tryptophan it Atlas (left) and Atsel barley (right) treated via the leaves (2a) and via the roots(2b). Dosage was 0.1 μmole per plant; treatment times were (left to right) 1 day, 4 days, and 14 days.

tinued movement occurred but was not prominent; and availability was low after the 4-day period. Apoplastic movement in treated leaves was medium to low (fig. XIV, 2*a*).

Movement from roots to tops from root application was lowest for tryptophan among the eight compounds tested; images were light in all cases; evidence for phloem movement was present only in the 1-day plant of Atlas barley. Accumulation in the roots themselves was high for all treatment times (fig. XIV, 2*b*); these plants resembled the 2,4,5-T treated plants described in chapter 8.

L-VALINE This amino acid showed the most similarity between leaf and root uptake (fig. XIV, 3*a*, *b*). Absorption and phloem transport via the leaves were medium; movement continued throughout the 1- and 4-day treatments; apoplastic movement in treated leaves was medium to heavy.

Movement of L-valine to the tops from root treatment was greatest of all the amino acids tested, and equal to or greater than was distribution from leaf treatment. Accumulation remained high in all roots throughout the 14-day period; redistribution from old to young leaves was prominent in the 1- and 4-day treatment periods; the tracer supply became exhausted toward the end of the 14-day treatment, but primary accumulation in the tips of the number two leaves was prominent in plants of both varieties.

DL-VALINE Phloem transport from leaf application was weakest in DL-valine-treated plants. Concentrations of tracer in untreated leaves was medium to light, and medium in roots. Apoplastic movement in treated leaves averaged medium; evidence for redistribution via phloem was lacking.

Evidence for xylem movement of DL-valine from roots to tops indicated medium transport in Atlas barley and low transport in Atsel. Accumulations in roots remained high, particularly in young roots and in growing root tips. Evidence for redistribution from old to young leaves was lacking.

Table XIV, 2, gives a summary of observations on the 4-day treatments of the Atlas and Atsel varieties. It is obvious that there are distinct differences in the amounts of amino acids moved, even though amino acids generally translocate from leaves via the phloem by symplastic move-

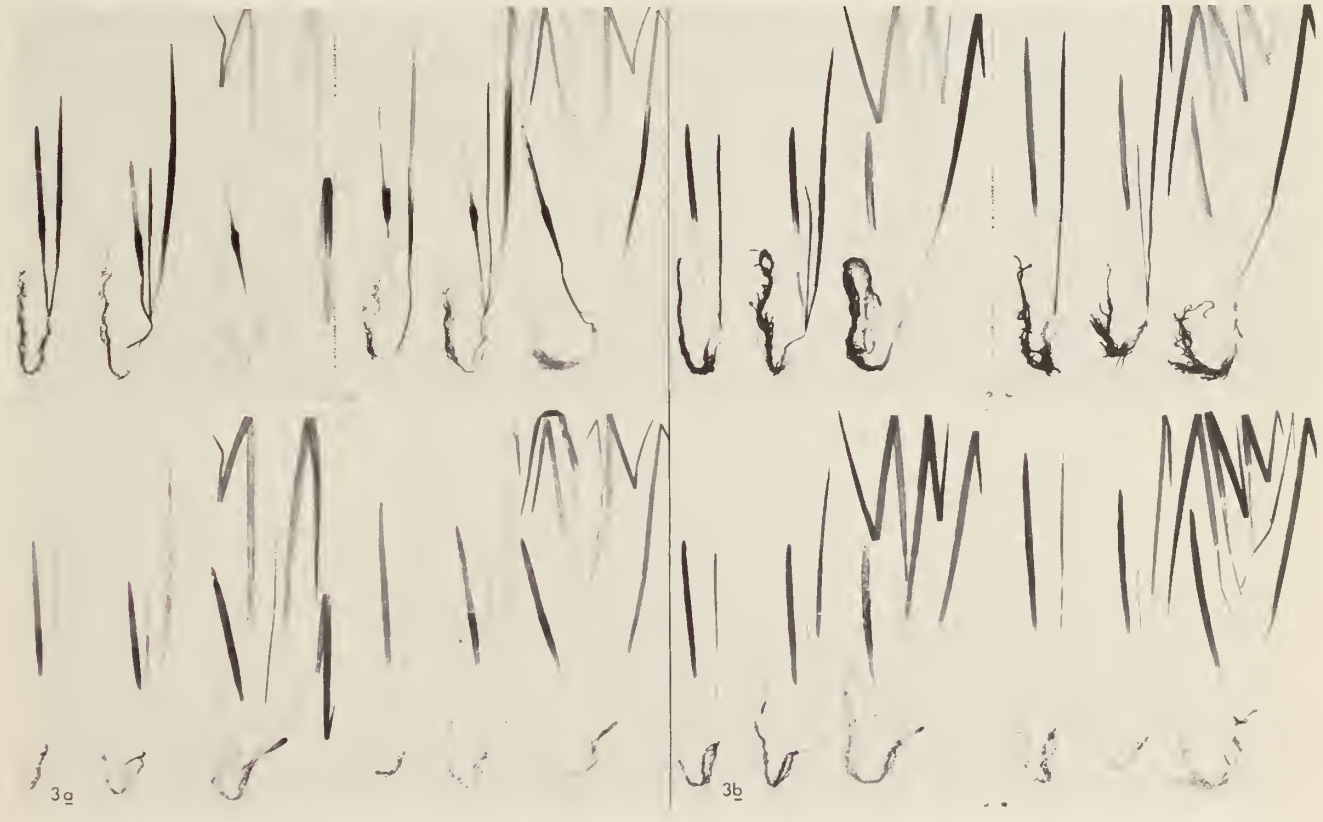


Fig.XIV, 3a, 3b. Distribution of L-valine* in Atlas and Atsel barleys treated via leaves (3a) and via roots (3b). Dosage was 0.1 μmole per plant; treatment times were (left to right) 1 day, 4 days, 14 days.

Table XIV, 2
Translocation Patterns of Eight Labeled Amino Acids
In Two Barley Varieties

Atlas barley, 4-day treatment

Amino acids	Leaf treatment; concentration in:				Root treatment; concentration in:				
	Treated leaf tip	Treated leaf base	Untreated leaves	Roots	Young leaves	Old leaves	Root tips	Mature roots	Σ †
DL-Arginine	+	+	++	+	++	+	+++	+	12
Glycine	++	+++	+++	+++	+++	+	+++	++	20
L-Histidine	++	++	+++	+++	+++	+	+++	++	19
DL-Lysine	++	++	+++	++	++	tr	+++	++	16
DL-Phenylalanine	+	+	++	++	++	+	+++	++	14
DL-Tryptophan	++	++	++	++	+	+	+++	+++	16
L-Valine	+	++	++	++	+++	++	+++	++	17
DL-Valine	+	+	+	+	+	+	+++	++	11

Atsel barley, 4-day treatment

DL-Arginine	+	+	++	+	++	+	+++	+	12
Glycine	++	++	+++	+++	++	+	+++	+++	19
L-Histidine	++	++	+++	+++	++	+	+++	++	18
DL-Lysine	+++	++	+++	+++	++	+	+++	+++	20
DL-Phenylalanine	+++	++	++	++	+	+	+++	++	16
DL-Tryptophan	++	+	++	++	+	tr	+++	+++	14
L-Valine	+	+	++	++	++	+	+++	++	14
DL-Valine	+	+	++	++	+	+	+++	++	13

Key to symbols: tr = trace, + = light gray, ++ = dark gray, +++ = black.
† Summation of plus signs

ment and from roots via xylem by apoplastic movement.

If the numbers of plus signs for each amino acid in table XIV, 2, are totaled as a rough measure of uptake and distribution for these 4-day treatments, the amino acids assume the following series in the two barley varieties:

Atlas	Atsel
Glycine	DL-lysine
L-histidine	Glycine
L-valine	L-histidine
DL-lysine	DL-phenylalanine
DL-tryotophan	DL-tryptophan
DL-phenylalanine	L-valine
DL-arginine	DL-valine
DL-valine	DL-arginine

Some amino acids are apparently more mobile than others in the phloem, some excel in xylem mobility, and there are over-all differences in mobility between the two varieties of barley. In general, greater quantities of these acids move about the plant following leaf application than they do following root application. Some amino acids act like 2,4-D inasmuch as they accumulate in roots and fail to move to tops in appreciable quantity; DL-valine and tryptophan are two such acids (fig. XIV, 2*b*). Others, such as L-valine, apparently move about as freely in the transpiration stream as they do via the phloem (fig. XIV, 3*a*, *b*).

Amino acids are normal products of plant metabolism and may be present in the assimilate stream in appreciable quantities (Mittler, 1957*b*). Probably a small fraction of the mobile assimilates at all times, they are known to move out of senile leaves in autumn in relatively large quantities, and thus it is not unexpected to find that they are quite mobile in the phloem. In xylem, however, simple nitrogen compounds are more often found in the amine and amide forms, and the rather free movement of L-valine from roots to tops of barley comes as a surprise. Because of the rather wide variation in xylem mobility between L-valine and tryptophan, these molecules should be examined carefully in hopes of finding some clue to the molecular configuration responsible for free xylem mobility in plants.

In regard to the xylem mobility shown in the dosage series of root treatments with tryptophan, it is interesting to note that the 0.05 µmole dosage in Atlas barley failed to enter the xylem and move up to the leaves, and that root accumulation was high. Since the movement at the higher dosages was normal it seems that there may be a threshold effect in this situation. This type of response is found with 2,4-D at injurious levels. However, there were no symptoms of injury in the case of the 0.1 µmole dosage shown in figure XIV, 2*b*, nor in the 0.2 µmole dosage shown in figure XIV, 1*b*. The avidity with which plant roots absorb, accumulate, and retain various compounds is an important phenomenon and one of great interest to people engaged in application of pesticides through the soil.

Chapter XV. Role of Formulation Additives in Absorption and Translocation of Herbicides

In the formulation of pesticides, carriers - such as water and oils - may largely determine the type of herbicidal action of a chemical. For example, if dinitro-ortho-cresol is dissolved in oil and applied to vegatation it has a general contact action, but when dissolved in water its sodium salt is highly selective. Surfactants also have important effects on spray action: sulfuric acid, a highly selective spray, may be made more general in its action by addition of Vatsol OT.

Since the introduction of 2,4-D and other systemic herbicides it has been observed that inclusion of surfactants appreciably increases the effectiveness of these materials. Figure XV, 1, shows the penetration of a fluorescent dye into the upper halves of two pear leaves having open stomata when Vatsol OT was included in the solution (upper leaves); where stomata were closed, no penetration took place (lower leaves). Recently, addition of solublizing compounds of a surfactant nature has been found to greatly increase the herbicidal activity of the substituted ureas. Because of these effects the authors made a study of surfactants in an effort to throw some light on the ways in which these compounds may alter the uptake, distribution, and toxic action of herbicides.

ROLE OF SURFACTANTS IN TRANSLOCATION

In many of the early studies on the absorption and translocation of tracer molecules it was observed that inclusion of a surfactant in the treatment solution enhanced the uptake of the tracer (Mitchell and Linder, 1950*a*, *b*). Although early thinking about this effect was almost entirely in terms of increased wetting, the concept gradually evolved that in some way penetration of the cuticle was being increased. Studies on the lowering of surface tension of water by surfactants proved that a limiting value was reached around 30 dynes per square centimeter, and that increasing surfactant concentration more than about 0.1 per cent by weight had little further effect on lowering surface tension. Results of field trials with 2,4-D, dalapon, and other herbicides indicated that concentration

Fig. XV, 1. Penetration of fluorescent dye through the stomata of pear leaves. Dye solution included the surfactant Vatsol OT at 0.5 per cent. Upper pair of leaves had open stomata; the lower pair had closed stomata. Treatment time, 2 minutes; apical halves of leaves immersed.

increases to approximately 1.0 per cent gave definite increases in herbicidal activity, particularly on perennial plants where both penetration and translocation were involved.

TWEEN 20 Jansen, Gentner and Shaw (1961) have made a comprehensive study of the effects of surfactants on herbicidal activity. Working with 63 surfactants, including cationic, anionic, nonionic, ampholytic and blended materials, and using these in conjunction with 2,4-D, dalapon, DNBP and amitrole on corn and soybeans, they found three characteristic types of action. Many surfactants caused progressive increase of herbicidal activity with logarithmic increases in concentration. Some surfactants caused a progressive supression of herbicidal activity with increase in concentration; others had no effect. Many surfactants showed marked phytotoxicity at high concentrations, and some were stimulating in the absence of herbicide at low concentration. Surfactant effects varied between the two species of test plants used and there were no obvious correlations between surfactant effects and surfactant structure. Jansen and his colleagues (1961) used Tween 20 (S-145), an anionic surfactant of very low phytotoxicity, as a standard.

Webster (1962) has studied the effect of Tween 20 on the entry of 2,4-D into leaves of *Kalmia augustifolia*. He found that entry rate of 2,4-D into young leaves decreased with an increase in Tween 20 of two-fold to eleven-fold for a given 2,4-D dosage; with a two-fold increase entry of 2,4-D into old leaves was increased.

In our early studies with labeled tracers we observed the increased wetting and penetration when 0.1 per cent Tween 20 was incorporated into the formulation of the treatment solution. In order to rule out wetting as a factor in our translocation studies, we used 1/10 per cent Tween 20 in all of our aqueous and 50 per cent alcohol treatment solutions (Weintraub *et al.*, 1950; Brown and Weintraub, 1950; Weintraub *et al.*, 1952*a*, *b*; Weintraub *et al.*, 1954).

Since we had used Tween 20 in most of our treatment solutions, it seemed advisable to study the effects of varying the surfactant concentration on the uptake of certain herbicides. Our first series of experiments used 2,4-D*; the second involved 2,4-D* and amitrole*.

The first experiment used 2,4-D* at dosages of 1/10 µmole, 4/10 µmole, and 8/10 µmole each, in 20 µl of solution. Tween 20 concentrations were 1/10 per cent and 4.0 per cent. Treatment times were 1 day and 4 days; plants were beans and barley. The only factors resulting in differences were concentration of 2,4-D, and time; intensity of labeling with 2,4-D* increased regularly through the concentration series and formative effects increased with both concentration and time. These responses were more apparent on bean than on barley, but the difference in Tween 20 concentration produced no effect visible in the autoradiographs of either plant.

A second experiment compared 2,4-D* and amitrole* at the same micromolar dosages as in the preceding experiment. Treatment time was 4 days; the plant was nasturtium. Again, there were no differences between the Tween 20 concentrations; the usual differences between 2,4-D* and amitrole* were expressed (fig. VIII, 2). Amitrole* was transported both symplastically and apoplastically; 2,4-D* distribution was entirely symplastic. Treatment in this experiment was to undersurfaces of leaves where there are numerous stomates. 2,4-D* caused the treated nasturtium leaf to turn yellow; amitrole* had no visible effect on the treated leaf within the 4-day treatment period.

A third experiment examined the role of Tween 20 on uptake of 2,4-D* and amitrole* by coleus plants (fig. XI, 3). Forty µl volumes of 2,4-D* and amitrole* treatment solutions of 0.005M concentration were applied to opposite leaves in the form of about twenty 2-µl droplets; the whole group of plants was covered by a polyethylene bag to maintain high humidity and was left for 4 days. Tween 20 concentrations were 1/10, 1.0 and 4.0 per cent. One leaf in each pair was treated on the upper surface, which was free of stomates, the other was treated on the lower surface where stomates were numerous.

Again there were no apparent differences between responses to different Tween

20 concentrations; with 2,4-D* there were marked differences between applications to upper and lower surfaces, but such differences were not apparent in the case of amitrole*. It is apparent that with 2,4-D* much more tracer entered through the lower leaf surface, as is shown by the strong labeling of a side shoot above the treated leaf and by similar labeling of the stem on the side of lower surface treatment. Translocation was strictly symplastic when 2,4-D* was used.

Amitrole* apparently moved through upper and lower leaf surfaces in about equal quantities; after entry there was considerable apoplastic movement, as indicated by the labeling of the untreated portions of the treated leaves and the intermediate-sized untreated leaves above the treated ones. But symplastic movement was also strong, as was accumulation in active sinks; this is apparent in the intense labeling of the young expanding leaves and of the buds at the upper end of and along the stem, and by the labeling of young growing roots. Old roots and stem tissue held an intermediate concentration of amitrole* - an indication of a lower accumulative avidity for this compound.

A test on fair-sized cotton plants, in which Tween 20 was incorporated at concentrations of 1/10 per cent and 4 per cent into 2,4-D* solutions of 2/10, 4/10, and 8/10 µmole dosages, likewise showed no observable differences in uptake or movement due to the surfactant.

A final test on the possible effect of Tween 20 on 2,4-D* absorption by soybean roots from the culture medium showed, if anything, a reduction in absorption between 1/10 per cent and 4 per cent Tween 20 concentration. The 2,4-D* was present at 1.10 µmole in 100 ml of Hoagland's solution.

These tests with unlabeled Tween 20 and labeled 2,4-D and amitrole apparently indicate that this surfactant has no role in the uptake and distribution of systemic herbicides. However, a large amount of observational data from the field indicates an enhancement of uptake by surfactants. Possibly, the growing of the plants in the greenhouse, the single-droplet application within a lanolin ring, or some other part of our experimental procedure was responsible for our negative results.

SODIUM LAURYL SULFATE One of the commonest of surfactants is sodium lauryl sulfate, an anionic surfactant which has long been readily available. When it was finally produced in the S^{35}-labeled form, we used some in our standard test. Applied to bean leaves at dosages of 1/10 µmole, 4/10 µmole and 8/10 µmole of treatment solution of 0.01 M concentration, with and without Tween 20 in the formulation, it showed no tendency to penetrate (fig. XV, 2, left). In bean, cucumber, and cotton it showed no apoplastic movement in the treated leaf; in soybean and barley there was slight apoplastic movement in an acropetal direction from the treated spot. Applied to roots via the culture medium at 1/10 µmole, 4/10 µmole and 8/10 µmole per 100 ml of solution, it moved slightly into bean in one day; labeling increased through the above dosage series. In cucumber and barley it was also moved in 1 day; in cotton and soybean it entered the roots but did not translocate to tops. In 4 days all the above species had faint to heavy labeling of the tops from root treatment (fig. XV, 2, 3, 4). In bean, where dosages were 1/10, 4/10, and 8/10 µmole per 100 ml, labeling increased in proportion to dosage (fig. XV, 2, right).

In leaf treatments, incorporation of Tween 20 at 1/10 per cent concentrations had no effect on penetration of sodium lauryl sulfate, nor did incorporation of unlabeled 2,4-D. When sodium lauryl sulfate-S^{35}-treated plants were extracted and the extracts chromatographed there was no evidence for movement of the S^{35} from the position of treatment on the leaf; extracts from roots and stems of root-treated plants produced spots indicating that the S^{35} remained near the origin. These spots correspond in Rf value to $SO_4^{=}$ion. S^{35} in sodium lauryl sulfate moved freely to the solvent front.

C. L. Foy, using S^{35}-labeled sodium lauryl sulfate alone and in conjunction with dalapon on leaves of cotton plants, found that this surfactant penetrated the cuticle and moved toward the leaf tip (apoplastic movement) but not out of the leaf via the phloem. After 7 days the S^{35} label was found throughout the plant; Dr. Foy considered it probable that this

Fig. XV, 2. Leaf treatment (left) and root treatment (right) of bean plants with S^{35}-labeled sodium lauryl sulfate at 0.4 μmole dosage for 4 days. The formulation was made up with 0.1 per cent Tween 20 in 50 per cent ethyl alcohol.

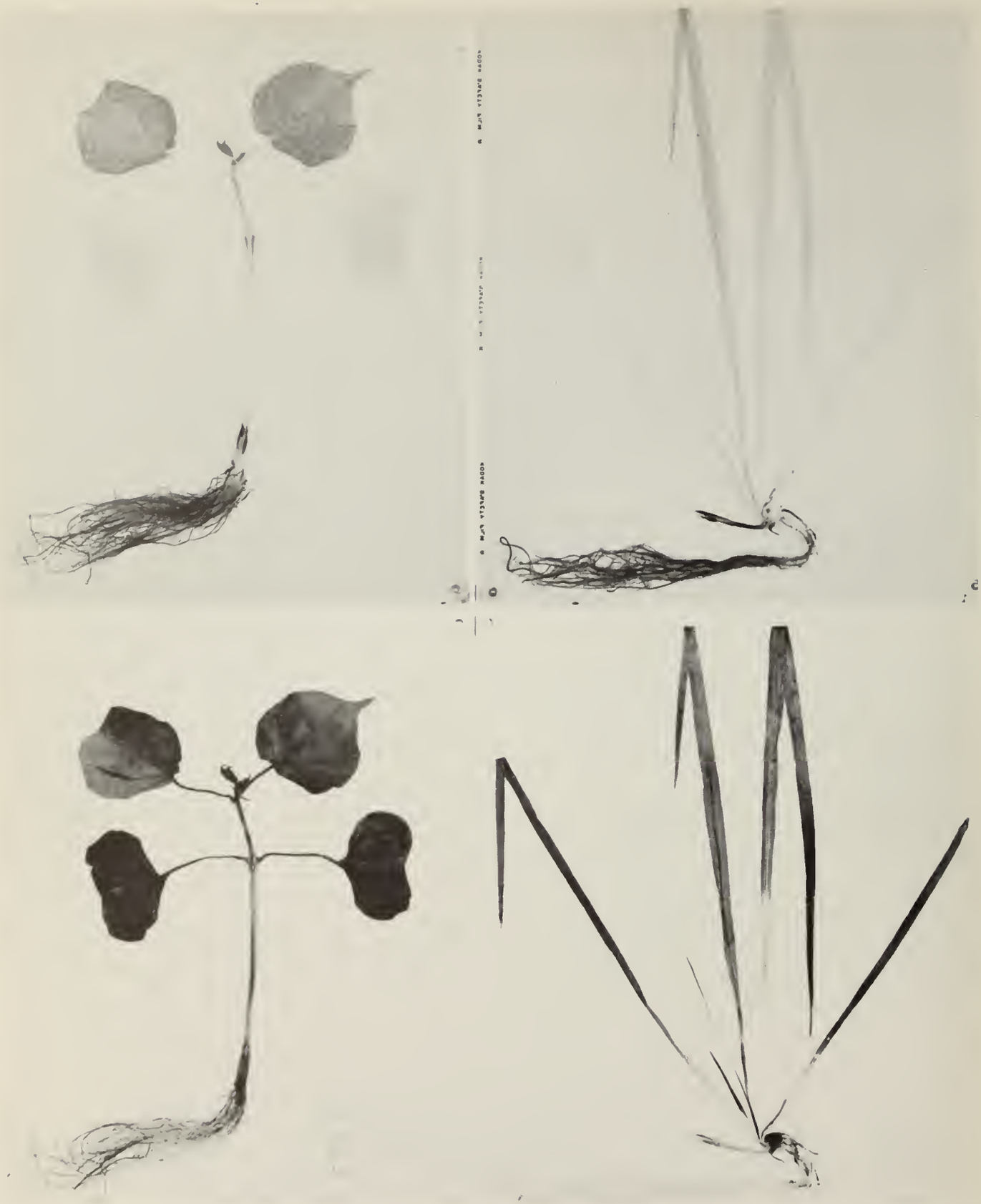


Fig. XV, 3. Movement of S^{35}-labeled sodium lauryl sulfate in cotton (left) and barley (right) from 4-day treatment via roots. Dosage was 0.1 μmole; the solution contained 0.1 per cent Tween 20.

Fig. XV, 4. Movement of S35-labeled sodium lauryl sulfate in soybean and cucumber.

was metabolized sodium lauryl sulfate, possibly sulfate ion.

Dr. Foy also studied uptake of T-1947-C^{14}, a surfactant of low phytotoxicity. This compound penetrated very slowly and moved only acropetally to the leaf tips; no C^{14} moved out of the leaf even in 7 days. Sodium lauryl sulfate-S^{35}, which was found to be intact in the leaf after 24 hours, moved predominantly in the veins; T-1947-C^{14} moved mostly in the interveinal regions. Moreover, T-1947-C^{14} accumulated in the lysigenous glands of the cotton leaf, as do the chloro- and methoxy-triazines; sodium lauryl sulfate-S^{35} did not do this.

These tests seem to indicate that sodium lauryl sulfate penetrated the leaf and was strongly retained in living cells in an altered form not mobile on chromatograph paper. To have enhancing action on a pesticide, such a material could act only in two ways - by increasing wetting, and by increasing penetration. Since the surfactant molecules did not move within the plant, it is difficult to see how they could affect the action of a chemical at a site removed from the point of application. Thus the action of the two surfactants so far tested must consist of an enhancement of uptake through increased penetration. The lack of effect noted in these tests, using droplet application to leaves of greenhouse-grown plants, must result from the conditions of the experiment. Droplet application in effect is a very different treatment from spraying, which may cover the total foliage surface with a liquid film.

Chapter XVI. Conclusions

Over 10 years of work with labeled tracers, using autoradiography as the principal tool, has resulted in pertinent contributions to man's understanding of fundamental plant physiology and of herbicidal action. It now seems certain that systemic distribution of foliage-applied compounds follows a source-to-sink pattern indicative of movement en masse of the assimilate stream via the phloem. The consistent bypassing of mature leaves, the high concentrations in young growing shoot tips, root tips, and intercalary meristems, and the reversibility of flow brought about by proper manipulation, all indicate a mass-flow type of mechanism. To best utilize such a mechanism the following conditions should be met:

a) For critical situations where systemic distribution of herbicides in perennial plants is desired, formulation of the phenoxy compounds in the emulsifiable acid or alkoxy aliphatic ester forms is essential.

b) Formulations and dosages should be chosen to avoid rapid contact toxicity to foliage, and to facilitate slow, ordered uptake. The dosage optimum for 2,4-D is lowest with light aliphatic esters, intermediate with amine salts, and higher with alkoxy esters and emulsifiable acid formulations.

c) Timing of application should be done on a physiological basis - that is, when leaf maturity, root growth and photosynthetic activity are all favorable.

d) Application procedures should meet the specific requirements of the weed being controlled. If there is a mixture of species, even the wisest compromise on procedures might not give satisfactory results, and repeated treatments may be necessary.

e) Activity of sink as well as source is essential; insufficient soil moisture for root growth may cause the most favorable formulation of phenoxy compounds to fail.

f) The use of amitrole, which is more mobile than 2,4-D and which does not require active root growth for response, may be the answer to many of the problems encountered with the phenoxy compounds. With amitrole, selectivity may constitute a problem, however, as some weed species are not susceptible to this herbicide.

Autoradiographic studies have shown that 2,4-D brings about a disruption of the vascular tissues of treated plants.

a) Where 2,4-D is being used, the dosage should be optimum; too high a dosage injures the source and too low a dosage will not kill the plant; too low a dosage may also block the phloem so that a repeat treatment may fail.

b) Where two or more herbicides are being used in combination, 2,4-D should be applied at the same time or later than the other material. If applied before, it stops movement of all materials into roots.

Comparative mobility studies on many labeled herbicides indicate that they move in the assimilate stream, and that entry into and removal from this stream are the principal factors affecting the amounts moved.

a) Requirements for translocation of the phloem mobile compounds are very much alike for all. Limitation of movement of the phenoxy compounds by accumulation or binding is substantial and can best be met by proper timing of application with respect to the physiological status of the plants. Plants reaching the optimum condition at different times should not be expected to respond favorably to a single common treatment.

b) Because of differing requirements for different herbicides, application times and methods should fit the chemical being used, the plants being sprayed, and the relevant physiological factors.

c) The need for understanding the physical nature of the translocation process, the chemical nature of the compounds translocated, and the special requirements for effective distribution, precludes the discovery by crude testing methods of new and revolutionary formulations or application procedures for using the well-known herbicides.

Different herbicide molecules move into plant roots at different rates. Some, such as the substituted ureas and symmetrical triazines, move through roots and into the tops quite rapidly; others, such as 2,4-D and maleic hydrazide, may move slowly.

a) Where 2,4-D is used as a preemergence treatment, it must be applied so as to contact the roots of germinating seedlings. Urea and triazine herbicides may successfully be applied to young seedling growth.

b) Formulation of 2,4-D for preemergence application in soil should allow for soil type and rainfall. The acid, low in solubility, is most persistent; the aliphatic esters are intermediate; the amine or sodium salts are least persistent.

c) The herbicide should come in contact with the emerging roots of the seedling weeds. In the absence of rainfall, soil incorporation is necessary.

The great differences in species susceptibility, observed in the use of herbicides in the field and confirmed by autoradiography, result mainly from differences in the reaction of living cells to herbicide toxicity at specific sites within the plant, not to differences in penetration or translocation.

a) Differences in formulation will bring about differences mainly in toxicity but not in selectivity. Selectivity of the newer foliar sprays, and of all soil-applied herbicides, is little affected by formulation.

b) If dosage is varied through a sufficient range, most herbicides are selective. Broad spectrum selectivity is most desirable; compounds having narrow selectivities are hazardous to use.

Autoradiographic studies have been carried out with labeled forms of the following herbicides:

2,4-D	Propazine
2,4,5-T	Prometone
Amitrole	Amiben
Maleic hydrazide	2,3,6-TBA
Dalapon	EPTC
Monuron	Barban
Simazine	Dacthal
2,4-DB	Alanap
NH_4SCN	Arsenate
Atrazine	PCP
Trietazine	Ammonium thiocyanate

2,4,5-T-butoxy-ethanol ester
Sodium trichloropropionate

Applied to a monocotyledonous test plant (barley) and a dicotyledonous test plant (kidney bean) through leaves and roots and given 1-, 4-, and 16-day test periods, these twenty-four compounds have provided a broad picture of the uptake, transport and redistribution of herbicides by plants. These studies give invaluable information for designing formulations and application methods for herbicides and coupled with studies on herbicidal breakdown in plants and soils they provide guidelines for the intelligent interpretation of residue data.

Autoradiography of whole treated plants will undoubtedly become more widely used as its advantages are increasingly recognized. The fact that treatment

normally consists of the application of microcurie amounts of a compound to an intact leaf, and that no further manipulation is needed during the normal treatment period, indicates the value of the method. Ready control of light, temperature, and humidity enables the researcher to study the effects of innumerable experimental conditions on the processes of absorption and translocation in plants. From our results to date we can see no effects of radiation *per se* on these processes; we conclude therefore that the labeled compounds used in our studies can be considered as nontoxic tracers so far as radiation effects are concerned.

With such a convenient method available there should be a bright future for studies using these tracer techniques. Additionally, a wide range of physiological investigations are indicated. Further studies on the division of labor between various plant organs (Bernard Forde, 1963) will undoubtedly challenge plant physiologists. More work on the basic aspects of solute absorption by roots and foliage should be carried on using autoradiography. And translocation studies aimed at a final resolution of the 100-year-old controversy over mechanism should be pursued.

When these studies have been made and the true mechanisms of transport processes have been elucidated, there remains the task of studying distribution patterns of inorganic nutrients, plant foods and agricultural chemicals in all of the major groups of plants. Only when knowledge from such studies is at hand will the plant physiologist and the agricultural consultant be able to prescribe fertilizer use, irrigation practices, hormone treatments and pesticide application with a satisfactory degree of certainty. Autoradiography will be one of the tools that will eventually enable the agriculturalist to bring on those increases in production of food, feeds, and fiber needed to provide for the world's expanding population.

Glossary of Terms

μ = micro

μg = microgram

μl = microliter

mc = millicurie

ml = milliliter

mm = millimeter

cm = centimeter

oz = ounce

MW = molecular weight

Rf = a term used in chromatography to designate the ratio of the distance moved by a solute to that moved by the solvent.

As^{77}, C^{14}, Ca^{45}, Cl^{36}, Fe^{59}, P^{32}, S^{35}, Zn^{65} = radioactive isotopes of the designated chemical elements.

* = radioactive. For example, 2,4-D* = radioactive 2,4-dichlorophenoxyacetic acid.

-C^{14} = compounds labeled with carbon-14. For example, $C^{14}O_2$ = carbon-14-labeled carbon dioxide; 2,4-D-C^{14} = carbon-14-labeled 2,4-D. Carboxy-carbon labeling was used in this work.

Cold 2,4-D = unlabeled 2,4-D

ADP = adenosine diphosphate

Alanap = N-1-naphylphthalamic acid

Amiben = 3-amino-2,5-dichlorobenzoic acid

Amitrole = 3-amino-1,2,4-triazole

ATP = adenosine triphosphate

Atrazine = 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine

Barban = 4-chloro-2-butynyl *N*-(3-chlorophenyl) carbamate

Dalapon = 2,2-dichloropropionic acid

Dacthal = 2,3,5,6-tetrachloroterephthalic acid, dimethyl ester

DNP = dinitrophenol

Duraset = N-meta-tolyl phthalamic acid

EPTC = ethyl N, N-di-*n*-propylthiol carbamate, Eptam

IAA = indole-3-acetic acid

MH = maleic hydrazide

Monuron = 3-(*p*-chlorophenyl)-1,1-dimethyl urea

Simazine = 2-chloro-4,6-bis (diethylamino)-*s*-triazine

TBA = 2,3,6-trichlorobenzoic acid

2,4-D = 2,4-dichlorophenoxyacetic acid

2,4-DB = 4-(2,4-dichlorophenoxy) butyric acid

2,4,5-T = 2,4,5-dichlorophenoxyacetic acid

2060 = ethyl, ethyl-*n*-butylthiol carbamate

1607 = *n*-propyl, di-*n*-propylthiol carbamate

2061 = propyl, ethyl-*n*-butylthiol carbamate

T-1947 = polyoxyethylene polyol, a nonionic surfactant

Tween 20 = polyoxyethylene sorbitan monolaurate, a nonionic surfactant of low phytotoxicity.

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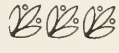

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